The effect of temperature on protein synthesis and growth efficiency in juvenile barramundi, *Lates calcarifer*

By

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Declaration

This thesis contains no material which has been accepted for a degree of diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the candidate's knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis

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Abstract

Temperature has a marked and direct effect on key physiological processes in fish, each species has a range of temperatures over which it survives (thermal tolerance range) and where growth occurs. Culture of barramundi, *Lates calcarifer*, is occurring at temperatures across the thermal tolerance (15-40°C). Consequently, this thesis examined the effect of temperature on protein metabolism: feed intake (FI, $g \cdot d^{-1}$), growth (SGR, $\% \cdot d^{-1}$), growth efficiency (in relation to protein and energy retention) and protein synthesis (k_s, $\% \cdot d^{-1}$) were measured. Fish were fed to satiation daily on the same diet. Protein synthesis was determined using the flooding dose method with ^{3}H-phenylalanine.

Models of FI, SGR and growth efficiency were developed from two experiments. The first examined temperatures from 21-33°C at 3°C intervals and the second looked at temperatures 27, 33, 36 and 39 °C. These models calculate the optimal temperature for each parameter to be 31°C. However, the optimal range for maximum growth efficiency (both PPV and PEV) is estimated to be 10 times wider than for FI or SGR. These models also predict an optimal temperature which is ~4°C higher than what has been previously determined for barramundi. The broad optimal range for growth efficiency is much more extensive than previously recognized for barramundi.

Experiments were conducted to quantify the effect of temperature on protein synthesis (k_s). The first experiment examined white muscle (WM) and whole body (WB) k_s from 21-33°C, at 3°C intervals, 24 h after feeding. No significant differences existed between temperatures, however regardless of temperature WBk_s was ~4 times greater than WMk_s. A second experiment was conducted at 21, 27 and 33°C on the WM, Liver and WBk_s at times 0, 4, 8, 12 and 24 h after feeding. At the 27 and 33°C WMk_s peaked 6 h after feeding and remained significantly elevated over the initial 12 h. A final experiment was conducted at 27, 33, 36 and 39°C on the WM, Liver, stomach, digestive tract and WBks at times 0, 2, 4, 6, 8, 12, and 24 h

after feeding. No differences occur between WBk_s at 27 and 33°C and peaks occur ~3 h after feeding; liver k_s peaks 2 h after feeding and is an order of magnitude higher than WBk_s. At 21 and 39°C, WBk_s is not affected by feeding and this corresponds to the low growth rates observed at these temperatures. The results of the three protein synthesis experiments have been combined with protein growth data to model protein turnover over the thermal tolerance range for juvenile barramundi.

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ASE	anabolic stimulation efficiency (%)
BW	body weight
CP	crude protein
Cs	RNA to protein ratio (mg RNA·g protein^{-1})
DO	dissolved oxygen
FER	feed efficiency ratio (g·g^{-1})
FI	feed intake
GE	gross energy
k_c	fraction rate of protein intake (%·d^{-1})
k_d	fraction rate of protein degradation (%·d^{-1})
k_g	fraction rate of protein growth (%·d^{-1})
k_{RNA}	RNA activity (k_s·g^{-1} RNA·d^{-1})
k_s	fraction rate of protein synthesis (%·d^{-1})
PER	protein efficiency ratio (g·g^{-1})
PEV	productive energy value (%)
PPV	productive protein value (%)
[RNA]	concentration of RNA (µgRNA·mg^{-1})
S_a	free pool specific radioactivity of ^{3}H-phenylalanine (dpm·nmol^{-1})
S_b	protein bound specific radioactivity of ^{3}H-phenylalanine (dpm·nmol^{-1})
SDA	specific dynamic action
SGR	specific growth rate (%·d^{-1})
SRE	synthesis retention efficiency (%)
WB	whole body
WM	white muscle

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

This thesis describes research into the protein metabolism of juvenile barramundi, *Lates calcarifer* Bloch 1790, in relation to temperature. Temperature is the key environmental parameter influencing the physiology of ectotherms. Experiments aimed to examine the feed intake, growth, growth efficiency and protein metabolism at temperatures (21-39°C) which span the thermal tolerance of barramundi. Protein synthesis, a fundamental process of growth, is energetically expensive and the efficiency of protein metabolism will be a key factor determining the growth efficiency of fish. Barramundi provide an excellent model species for this research, not only do they have a wide thermal tolerance, they are a fast-growing robust species, a commercially important aquaculture species worldwide and are being cultured at temperatures which span the thermal tolerance range. The aim of this chapter is to provide a background on the effects of temperature on feed intake, growth and protein synthesis in fish.

1.2 TEMPERATURE EFFECTS ON FEED INTAKE AND GROWTH

Temperature has a marked and direct effect on the physiology of ectothermic animals, including fish species and is considered the most important environmental factor which will affect growth of fish (Brett and Groves, 1979; Elliott, 1994). Every fish species has a range of temperatures where survival and growth occurs, this is the thermal tolerance range. Within this temperature range, is an optimal temperature range where maximum feed intake and growth occur (Elliott, 1994). The relationship between these two parameters, expressed as growth efficiency, is also maximized over an optimal temperature range (Brown, 1957; Elliott, 1994; Jobling, 1994). It is of interest to consider the thermal range for different parameters, particularly growth efficiency and protein turnover

At the lower end of the thermal tolerance range, feed intake and growth is low. As temperature increases, feed intake and growth increase (Brown, 1957; Jobling, 1997). These two parameters do not increase at the same rate due to an exponential increase in metabolism. As temperature reaches the optimum, feed intake will increase to a maximum. As temperature continues to increase past the optimum temperature a rapid decrease in feed intake will occur (Jobling, 1997). The growth of fish follows a similar pattern as temperature increases, however, as temperature progresses past the optimum temperature range growth rate decreases at a slower rate than feed intake. The temperature for maximum feed intake will generally be higher than that for optimal growth (Jobling, 1994). As temperature increases the metabolic rate increases (Fry, 1957) and at any given temperature the difference between the feed intake and the metabolic rate will determine the amount of energy available for growth (Jobling, 1994). At low temperatures, growth is limited by feed intake. At high temperatures, the metabolic rate can no longer be sustained by the feed intake, and the growth of the fish is compromised (Jobling, 1997).

As stated above, temperature has a demonstrated effect on the growth of fish due to changes in feed intake and metabolic rate. Some evidence has shown that under changing temperatures, growth rate can be increased, however this is generally only the case when fluctuations are small and within the optimal temperature range for fish (reviewed by Jobling, 1997). However, sharp increases in temperature induce an abnormal increase in appetite (Kestemont and Baras, 2001), this is due to a rapid increase in metabolic rate and fish are know to continue to feed in order to meet energetic needs (Wilson, 2002). An added benefit of a variable thermal regime is that fish will then generally tolerate higher temperatures (reviewed by Jobling, 1997). The tolerance fish have to changing or stable temperatures can also be dependant upon the size of the fish with smaller fish being more affected by changing water temperatures than larger fish (Elliott, 1994).

Various models have been used by different researchers to examine the relationship between temperature and feed intake and growth in fish, (Elliott, 1982; Elliott and Hurley, 1998; McCarthy, *et al.*, 1998; Bjornsson, *et al.*, 2001; Forseth, *et al.*, 2001). Elliott and Hurley (1998) developed a new functional model in relation to biological conditions, such as a wide range of constant temperatures, fish size and ration levels. Other researchers have utilized polynomial models to describe these relationships (Imsland, *et al.*, 1996; McCarthy, *et al.*, 1998; Person-Le Ruyet, *et al.*, 2004). It is generally accepted that there are limitations to using mathematical models to describe biological systems. For example, in the rate-temperature curves described by Jobling (1994) the steepness of the rate decline above the optimal temperature would be underestimated using a quadratic polynomial due to the symmetry of the model. Despite these limitations this method was employed in this thesis as it was felt that that this model best describe the relationships to be presented here.

1.2 PROTEIN SYNTHESIS

Clearly, protein synthesis is an essential part of growth in both endothermic and ectothermic animals and has a considerable impact on metabolism (reviewed by Houlihan, 1991; Houlihan, *et al.*, 1995b;1995a; Carter and Houlihan, 2001). In juvenile fish, when rapid growth is occurring, protein synthesis has been shown to account for up to 42% of the energy expenditure (Houlihan, *et al.*, 1988). This energetic cost is mainly associated with the process of mRNA translation (see protein turnover section) which is stimulated by feed intake and can be correlated with oxygen consumption in fish (Houlihan, 1991). Ultimately the cost of growth will be dependant upon the cost of protein turnover, however, there are many pathways of protein degradation of which, relatively few (along with their associated costs) have been clearly determined in fish (reviewed by Hawkins, 1991). Although recent research is starting to address this (Martin, *et al.*, 2001; Dobly, *et al.*, 2004).

Protein synthesis occurs in all tissues at varying rates within animals and each tissue contributes differently to whole body protein synthesis. The liver has the highest rates followed by the gill, digestive tract, and the spleen (Houlihan, *et al.*, 1986; 1988) and the white muscle has the lowest fractional rates in fish (Houlihan, *et al.*, 1988). The liver is central to protein metabolism and contributes a large proportion of the whole body protein synthesis (reviewed by Carter and Houlihan, 2001), however, the white muscle is the largest tissue mass in fish and is therefore the most representative of the whole body protein synthesis. Thus, the relationship between the white muscle and whole body protein synthesis is significant and linear with the whole body having 2-4 times the rate of white muscle protein synthesis (Carter and Houlihan, 2001). Due to the ease of measuring white muscle in comparison to whole body, there is considerable interest in this relationship to predict whole body protein synthesis (reviewed by Carter and Houlihan, 2001). For example, a general linear relationship between these two rates has been developed using data from Atlantic cod, rainbow trout and wolffish (Carter and Houlihan, 2001).

Protein synthesis is stimulated in all organs and tissues by feeding (reviewed by Jobling, 1983; Houlihan, 1991; Houlihan, *et al.*, 1995a; Carter and Houlihan, 2001; McCue, 2006) resulting in a post-prandial increase which is part of specific dynamic action (SDA). SDA is the increase in metabolic rate due to the processes associated with feeding and digestion as well as the biochemical processes involved in the post absorptive state (such as protein synthesis, reviewed by McCue, 2006) . SDA is generally quantified by measurements of post-prandial oxygen consumption (Houlihan, 1991). The pattern of post-prandial protein synthesis has been shown to follow the pattern of SDA with a rapid rise occurring shortly after feeding followed by a slow return to the pre-feeding rate (Lyndon, *et al.*, 1992). The post-prandial peak of protein synthesis occurred at different times for different tissues in rainbow trout (McMillan and Houlihan, 1989). After a 6 d fast, the liver protein synthesis peaked 3 h after feeding while the white muscle peaked at 6 h. The rapid response of liver protein synthesis to feed intake is attributed to its central role in protein

metabolism in order to regulate the dietary amino acid influx to maintain a relatively stable amino acid pool (Houlihan, *et al.*, 1995b), while peak rates in white muscle occur later and at a lower lever resulting in whole body SDA being a fusion of the individual tissue responses to feed intake (reviewed by Houlihan, 1991). It has been suggested that liver protein synthesis as the underlying factor of SDA (Jobling, 1981) due to the high metabolic costs associated with protein synthesis (reviews by Jobling, 1981; Houlihan, *et al.*, 1995b;1995a; Carter and Houlihan, 2001; McCue, 2006) and the liver accounting for ~31% of whole body protein synthesis (Lyndon, *et al.*, 1992).

Different techniques have been used to measure protein synthesis in fish, with each method having merits for specific applications. The major methods used in fish physiology are the flooding dose method (Garlick, *et al.*, 1980), constant infusion (Haschemeyer, et al., 1979) and end-point analysis (Carter, *et al.*, 1994). The flooding dose method causes a large influx of radioactive amino acid to flood the amino acid pools and incorporation to occur at all levels, from cellular to organismal (reviewed by Garlick, *et al.*, 1994; Houlihan, *et al.*, 1995b). Constant infusion of a radioactive amino acid can provide long-term measure of protein synthesis (days to weeks) and has been calculated from blood plasma and skin mucus levels (Fauconneau and Tesseraud, 1990). This technique is difficult in fish due to the intricacies of administering the tracer to fish over periods of time (Haschemeyer and Smith, 1979; Fauconneau and Tesseraud, 1990) and therefore is more often used in mammalian studies. The end-point analysis uses a stable isotope which attaches to the end product of protein metabolism (i.e. ammonia) and provides whole body rates of protein synthesis. This method accounts for post-prandial fluctuation, can be administered without handling the animals and repeated measurements are possible (reviewed by Houlihan, *et al.*, 1995b; Carter and Houlihan, 2001). This method is also beneficial in situations where using a radioactive label is not possible (Carter, *et al.*, 1998). However, the flooding dose method is applicable to different tissues if making within day measurements. For these reasons it was selected for use in the present study.

The various approaches and their merits for measuring protein synthesis have been the subject of discussion among researchers (Garlick, *et al.*, 1994; Rennie, *et al.*, 1994; Caso, *et al.*, 2006) and have recently been addressed in human muscle protein synthesis, flooding dose and constant infusion techniques gave analogous results (Caso, *et al.*, 2006). It was also suggested that the method used should be the most suitable for the experimental design and chosen for the ease of analysis. A further reason the flooding dose method was chosen for the experiments in this thesis, this method had the advantage that the flooding dose becomes stable after minutes as compared to hours for the constant infusion method (Garlick, *et al.*, 1994) it is therefore most suitable for determining short term protein synthesis rates with 24 h in both tissues and the whole fish and can be used in small fish. The flooding dose method (Garlick, *et al.*, 1980) uses a single injection of ^{3}H-phenylalanie to flood the amino acid free pools. All tissue free pools are flooded to a similar level. The various rates of protein synthesis in different tissues will affect the time that the flooding dose remains at a constant level. It is therefore important to examine the specific radioactivity over time of both the free pool and the bound ^{3}H-phenylalanine. Furthermore, when considering the effects of temperature on the free pool and incorporation of ^{3}H-phenylalanine it is crucial to look at these relationships given that temperature can alter the free pool (Foster, et al., 1992). The method depends on the assumption that the free pool remains constant while the incorporation of ^{3}H-phenylalanine increases in a linear fashion over the incorporation time (Garlick, et al., 1980). However, biological variables such as different feed intakes and growth rates between fish introduce a relatively high level of variation into incorporation data.

1.3 PROTEIN TURNOVER

Protein turnover is a continuous cycle consisting of protein synthesis, protein degradation with the net result being protein accretion when synthesis is greater than degradation. Protein turnover is generally measured by combining measurements of

protein synthesis and protein growth thereby obtaining an estimate for the much more complicated measurement of protein degradation by calculating the difference of the two. This means comparing long-term growth data (over days to weeks or months) with short term protein synthesis data (obtained over hours) to determine protein degradation. This can often be misleading when calculations of protein synthesis are taken from a single measure and not necessarily accounting for the daily fluctuations which occur from feeding (reviews by Houlihan, 1991; Carter and Houlihan, 2001) or possibly diurnal rhythms (Houlihan, *et al.*, 1995b). Other measures of protein turnover include a series of relationships between protein synthesis, protein consumption and protein growth (reviewed by Houlihan, *et al.*, 1995b). Anabolic stimulation efficiency (ASE) relates protein consumption and protein synthesis and is a measure of capacity of protein metabolism. Synthesis retention efficiency (SRE) is the proportion of synthesized proteins which are retained as growth, this measure has been shown to be inversely related to ASE (Mathers, *et al.*, 1993). Many factors (including temperature) have been shown to affect the measures of protein turnover. The cycle of protein turnover allows animals to adapt to changing temperatures and stressful environments which can damage proteins, which can be degraded and replaced through protein turnover (reviewed by Houlihan, *et al.*, 1995b). This process occurs through the degradation of the damaged proteins and the synthesis of new proteins. The retention of synthesized proteins will increase with temperature (reviewed by Houlihan, *et al.*, 1995b) and in larval nase, *Chondrostoma nasus,* protein degradation was not affected by increasing ration however protein growth was significantly increased (Houlihan, *et al.*, 1992). A key strategy in protein metabolism is for an animal to have high SRE, indicative of low protein degradation, minimized turnover and high growth. A high ASE is related to high protein turnover (i.e. high protein degradation) and therefore the fish must recycle the protein which are being synthesized, an energetically expensive process. Dietary protein, energy and amino acid balance will also have a direct effect on the feed intake and consequently the protein metabolism in fish (Carter and Houlihan, 2001).

The mechanism driving protein synthesis is defined as mRNA translation and consists of three distinct phases: initiation, elongation and termination. The process requires an initial charge of tRNA with a specific amino acid. Initiation combines the charged tRNA with mRNA and ribosome to form the initiation complex, peptide chains are then formed through the accumulation of charged tRNA's on the mRNA template. This continues until a stop codon is reached within the mRNA (Taylor and Brameld, 1999). Protein synthesis is the most energetically costly component of protein turnover and this highlights the importance of RNA in the process of protein turnover. It has been estimated that 40 mmol of ATP are required to synthesize one gram of protein (reviewed by Carter and Houlihan, 2001). RNA is generally expressed with its relationship to protein and by RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA$\cdot d^{-1}$). The ratio of RNA to protein (Cs:RNA:protein) is often termed the capacity of protein synthesis and through increasing this ratio and increasing k_{RNA} offers a means to increase protein synthesis (Carter and Houlihan, 2001). At sub-optimal temperatures, increases in RNA concentrations with lower k_{RNA} have been established as a mechanism to maintain similar protein synthesis rates as fish at optimal temperatures (Foster, et al., 1992; McCarthy, et al., 1999).

1.4 TEMPERATURE EFFECTS ON PROTEIN TURNOVER

The patterns of physiological processes in relation to temperature are well established and outlined above (Brown, 1957; Fry, 1957; Jobling, 1994; 1997). It is in question whether protein synthesis follows a similar asymmetric response to temperature. To date, this has only been shown in isolated hepatocytes from rainbow trout (Pannevis and Houlihan, 1992). White muscle and liver protein synthesis data for other animals (reviewed by McCarthy and Houlihan, 1997), show a comparable linear response to temperature meaning that both the liver and white muscle respond to temperature in a similar manner, eliciting a single temperature effect from all tissues regardless of the obvious differences in the rates of protein synthesis (reviewed by McCarthy and Houlihan, 1997). As yet, no study on fish has measured temperatures which span

across the thermal tolerance range to determine weather the response to temperature is truly linear or asymmetric.

The influence of temperature on protein synthesis is further complicated by the stimulation of protein synthesis by feeding. A large question surrounds how to account for these differences in feed intake and therefore protein synthesis due to temperature. Foster *et al.* (1992) addressed the issue of acclimatization vs. acclimation. Acclimatization is the changing of one parameter (water temperature) and as a result changing another (feed intake when fish are fed *ad libitum*), where acclimation is the changing of only one parameter and all other factors remaining the same (feeding fish the same ration). With acclimation the fish feed at the same rate and have similar protein synthesis rate at different temperatures (Foster, et al., 1992). In the majority of research fish are acclimatized, feeding *ad libitum* rations at different temperatures (reviewed by McCarthy and Houlihan, 1997). This allows the differences in protein synthesis to be determined and still consider the different mechanisms that fish use to alter protein synthesis rates (reviewed by McCarthy and Houlihan, 1997). This coupled with protein growth data would provide valuable information on protein growth efficiency especially for larval and small juvenile fish, where fast growth (and high efficiency) is essential for survival. This was the approach taken in this thesis.

1.5 BARRAMUNDI

Barramundi is a catadromous species naturally distributed across northern Australia and extend up to Southeast Asia to the Persian Gulf. Barramundi is a robust species with a wide thermal tolerance (15-40°C) and are commercially cultured in temperatures ranging from 22-35°C (Tucker, *et al.*, 2002) in both intensive and extensive systems over the full range of salinities (Tucker, *et al.*, 2002; Rimmer, 2003). They are a fast-growing species and a marketable product can be obtained in under 1 year. Their wide thermal tolerance has allowed the barramundi aquaculture

industry to rapidly expand. This expansion has occurred to areas outside its natural range from southern Australia to northeastern Unites States and northern Europe (Carter, *et al.*, In press). Since 1984, worldwide commercial culture has increased more than 17 times to nearly 30,000 t (FAO, 2006) and within Australia culture has steadily increased and this trend is expected to continue. Barramundi was selected as a model species for these experiments because it is a robust, fast-growing species which can tolerate a wide range of environments (Carter, *et al.*, In press). In addition, the temperature issues addressed in this thesis are pertinent to the expanding industry where barramundi are being cultured in temperatures across the thermal tolerance for the species (Tucker, *et al.*, 2002).

1.6 AIMS OF THIS STUDY

Information on the effect of temperature on barramundi growth is limited and to date, no work on the effects of temperature on protein metabolism has been performed. This study focused on examining the feed intake, growth, growth efficiency and protein synthesis across the thermal tolerance range (21-39°) and addressed the following aims:

- To determine the optimal temperature for feed intake, growth and growth efficiency for juvenile barramundi
- To measure the effect of temperature on post-prandial and daily rates protein synthesis.
- To determine the optimal temperature for protein turnover in juvenile barramundi
- To model feed intake, growth, growth efficiency and protein turnover at temperatures which span the thermal tolerance for barramundi.

1.7 NOTES ON THIS STUDY

The experimental chapters (Chapters 2-6) in this thesis are presented in manuscript format for publication in peer-reviewed journals. Consequently, some of content of this thesis may be repeated, in particular the introduction and material and methods.

Chapter 3 has already been published as:

Katersky, R.S. and Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer,* at high temperatures. Aquaculture 250, 775-780.

Chapter 4 models feed intake, growth and growth efficiency data from Chapters 2 and 3 over the temperature range of 21-39°C.

Chapter 7, the general discussion uses protein synthesis and protein growth data from Chapters 1, 2, 5 and 6 to create a series of linear regression to examine the relationships between measures of protein turnover across all temperatures. Due to low feed intake on the day of the flooding dose in Chapter 6, growth rates for these data have been calculated from the linear regression of k_c and k_g in order to create models of protein turnover over the temperature range of 21-39°C. These models are based on the actual protein synthesis measured.

1.8 REFERENCES

Bjornsson, B., Steinarrson, A., Oddgeirsson, M., 2001. Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). ICES Journal of Marine Science 58, 29-38.

Brett, J.R., Groves, T.D.D., 1979. Physiological Energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Vol. VIII: Bioenergetics and Growth. Acedemic Press, New York, NY, pp. 279-351.

Brown, M.E., 1957. Experimental studies on growth. In: Brown, M.E. (Ed.), The Physiology of Fishes Vol. I. Academic Press, New York, N.Y., pp. 361-400.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Wright, P., Anderson, P. (Eds.), Fish Physiology, Vol. 20: Nitrogen Excretion. Academic Press, New York, NY, pp. 31-75.

Carter, C.G., Glencross, B., Katersky, R.S., Bermudes, M., In press. Centropomidae Latidae. In: Le Francois, N.R., Jobling, M., Carter, C.G., Blier, P.U. (Eds.), Fish species selection for intensive aquaculture diversification. CAB International, Wallingford, CT.

Carter, C.G., Seeto, G.S., Smart, A., Clark, S., van Barneveld, R.J., 1998. Correlates of growth in farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castelnau). Aquaculture 161, 107-119.

Carter, C.G., Owen, S.F., He, Z.-Y., Watt, P.W., Scrimgeour, C., Houlihan, D.F., Rennie, M.J., 1994. Determination of protein synthesis in rainbow trout, *Oncorhyncus mykiss*, using a stable isotope. Journal of Experimental Biology 189, 279-284.

Caso, G., Garlick, P.J., Ballou, L.M., Vosswinkel, J.A., Gelato, M.C., McNurlan, M.A., 2006. The increase in human muscle protein synthesis induced by food intake is similar when assessed with the constant infusion and flooding techniques. Journal of Nutrition 136, 1504-1510.

Dobly, A., Martin, S.A.M., Blaney, S.C., Houlihan, D.F., 2004. Protein growth rate in rainbow trout (*Oncorhynchus mykiss*) is negatively correlated to liver 20S proteasome activity. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 137, 75-85.

Elliott, J.M., 1982. The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comparative Biochemistry and Physiology Part B 73B, 81-91.

Elliott, J.M., 1994. Growth and energetics of brown trout. In: Elliott, J.M. (Ed.), Quantitative ecology and the brown trout. Oxford University Press, Oxford, pp. 69-102.

Elliott, J.M., Hurley, M.A., 1998. A new functional model for estimating the maximum amount of invertebrate food consumed per day by brown trout, *Salmo trutta*. Freshwater Biology 39, 339-349.

FAO Fishery Information Data and Statistics Unit, F., 2006. Fishery Statistical Collections. FIGIS Data Collection. FAO - Rome. Updated Thu Sep 21 14:02:30 CEST 2006. http://www.fao.org/figis/servlet/static?dom=collection&xml=global-aquaculture-production.xml (Accessed Oct 6 2006).

Fauconneau, B., Tesseraud, S., 1990. Measurement of plasma leucine flux in rainbow trout (*Salmo gairdneri* R.) using osmotic pump. Preliminary investigations on influence of diet. Fish Physiology and Biochemistry V8, 29-44.

Forseth, T., Hurley, M.A., Jensen, A.J., Elliott, J.M., 2001. Functional models for growth and food consumption of Atlantic salmon parr, *Salmo salar*, from a Norwegian river. Freshwater Biology 46, 173-186.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod. Canadian Journal of Zoology 70, 2095-2102.

Fry, F.E.J., 1957. Aquatic respiration of fish. In: Brown, M.E. (Ed.), The Physiology of Fishes. Academic Press, New York, N.Y., pp. 1-63.

Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissue by injection of [^{3}H] phenylalanine. Biochemical Journal 192, 719-723.

Garlick, P.J., McNurlan, M.A., Essem, P., Wernerman, J., 1994. Measurement of tissue protein synthesis rates in vivo: a critical analysis of contrasting methods. American Journal of Physiology 266, E287-E297.

Haschemeyer, A.E., Persell, R., Smith, M.A.K., 1979. Effect of temperature on protein synthesis in fish of the Galapagos and Perlas Islands. Comparative Biochemistry and Physiology Part B, 91-95.

Haschemeyer, A.E.V., Smith, M.A.K., 1979. Protein synthesis in liver, muscle and gill of mullet (*Mugil cephalus* L.) in vivo. Biological Bulletin 156, 93-102.

Hawkins, A.J.S., 1991. Protein turnover:a functional apprasial. Functional Ecology 5, 222-233.

Houlihan, D.F., 1991. Protein turnover in ectotherms and its relationship to energetics. In: Gilled, R. (Ed.), Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin Heidelberg, pp. 1-43.

Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effects of body size. Physiological Zoology 59, 482-493.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995a. Protein synthesis in fish. In: Hochachka, P., Mommsen, P. (Eds.), Biochemistry and Molecular Biology of Fishes, pp. 191-220.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995b. Protein turnover in animals. In: Walsh, P.J., Wright, P. (Eds.), Nitrogen metabolism and excretion. CRC Press, New York, pp. 1-32.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45, 951-964.

Houlihan, D.F., Wieser, W., Foster, A., Brechin, J., 1992. *In vivo* protein synthesis rates in larval nase (*Chondrostoma nasus* L.). Canadian Journal of Zoology 70, 2436-2440.

Imsland, A.K., Sunde, L.M., Folkvord, A., Stefansson, S.O., 1996. The interaction of temperature and fish size on growth of juvenile turbot. Journal of Fish Biology 49, 926-940.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. Journal of Fish Biology 18, 385-400.

Jobling, M., 1983. Towards an explanation of specific dynamic action (SDA). Journal of Fish Biology 23, 529-555.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Kestemont, P., Baras, E., 2001. Environmental factors and feed intake: Mechanism and Interactions. In: Houlihan, D.F., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell Science, Ltd., Oxford, pp. 131-156.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. Journal of Comparative Physiology B 162, 209-215.

Martin, S.A.M., Cash, P., Blaney, S., Houlihan, D.F., 2001. Proteome analysis of rainbow trout (*Oncorhynchus mykiss*) liver proteins during short term starvation. Fish Physiology and Biochemistry 24, 259-270.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., Burren, L.J., 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. Journal of Fish Biology 43, 245-263.

McCarthy, I., Houlihan, D.F., 1997. The effect of temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (*Salmo salar* L.) stocks in Europe as a result of global warming. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 51-77.

McCarthy, I., Moksness, E., Pavlov, D.A., 1998. The effects of temperature on growth rate and growth efficiency of juvenile common wolffish. Aquaculture International 6, 207-218.

McCarthy, I.D., Moksness, E., Pavlov, D.A., Houlihan, D.F., 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Sciences 56, 231-241.

McCue, M.D., 2006. Specific dynamic action: A century of investigation. Comparative Biochemistry and Physiology - Part A 144, 381-394.

McMillan, D.N., Houlihan, D.F., 1989. Short-term responses of protein synthesis to re-feeding in rainbow trout. Aquaculture 79, 37-46.

Pannevis, M.C., Houlihan, D.F., 1992. The energetic cost of protein synthesis in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). Journal of Comparative Physiology B 162, 393-400.

Person-Le Ruyet, J., Mahe, K., N., L.B., Le Delliou, H., 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquaculture 237, 269-280.

Rennie, M.J., Smith, K., Watt, P.W., 1994. Measurement of human tissue protein synthesis: an optimal approach. American Journal of Physiology 266, E297-E307.

Rimmer, M.A., 2003. Barramundi. In: Lucas, J.S., Southgate, P.C. (Eds.), Aquaculture: Farming Aquatic Animals and Plants. Blackwell Publishing, Oxford, England, pp. 364-381.

Taylor, P.M., Brameld, J.M., 1999. Mechanisms and regulation of transcription and translation. In: Lobley, G.E., White, A., MacRae, J.C. (Eds.), Protein metabolism and nutrition. Wageningen Pers, Wageningen, pp. 25-50.

Tucker, J., J.W., , Russel, D.J., Rimmer, M.A., 2002. Barramundi Culture: A success story for aquaculture in Australia, World Aquaculture, pp. 53-59.

Wilson, R.P., 2002. Amino acids and proteins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Pres, San Diego, pp. 144-179.

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CHAPTER 2

GROWTH AND PROTEIN SYNTHESIS OF JUVENILE BARRAMUNDI, *LATES CALCARIFER*, AT DIFFERENT TEMPERATURES

2.1. ABSTRACT

Temperature is recognized to be the most important environmental factor affecting growth and protein synthesis in fish. The optimal temperature for growth of juvenile barramundi is 31°C, although culture often occurs at temperatures which are above and below this optimum. Juveniles (2.96 ± 0.46 g) were held at five different temperatures ranging from 21 to 33°C at 3°C intervals. Fish were fed to satiation twice daily (504.5 $g \cdot kg^{-1}$ crude protein, 190.5 $g \cdot kg^{-1}$ lipid, 128.5 $g \cdot kg^{-1}$ ash, 20.2 $GE \cdot MJ\ kg^{-1}$). Daily feed intake (g), growth ($\%\cdot d^{-1}$), growth efficiency, protein synthesis (measured 24 h after feeding) were determined for each temperature. Feed intake was significantly higher at 33°C, than at any other temperature. Growth and growth efficiency were not significantly different between the 27, 30 and 33°C groups but were significantly higher than the 21 and 24°C groups. Growth efficiency remains optimal over a large temperature range, from 27 to 33°C. Protein synthesis at 24 h after feeding was not significantly different between temperatures, however, whole body protein synthesis was 4 times the white muscle protein synthesis, irrespective of temperature. Protein synthesis measured at 24 h after feeding gave an underestimated daily protein synthesis and degradation, by missing the post-prandial peak.

Keywords: Asian sea bass; Barramundi; Growth; *Lates calcarifer;* Protein synthesis; Temperature

2.2. INTRODUCTION

Temperature has been identified as the most important abiotic factor affecting growth rate in ectotherms, including fish (Brett and Groves, 1979). Temperature has a direct effect on feed intake and metabolism, including protein turnover, and therefore on the growth efficiency of fish (Jobling, 1994). As temperature increases across the thermal tolerance of a species the feed intake and growth follow asymmetric patterns, increasing to a maximum at the optimal temperature (Jobling, 1997; McCarthy and Houlihan, 1997). As temperature increases above the optimum, there is a sharp decrease in both of these parameters and therefore in growth efficiency. Metabolism increases exponentially as temperature increases and at any given temperature, the difference between the feed intake and metabolic rate will determine the energy available for growth of the organism (Brett and Groves, 1979; Jobling, 1994). There have been numerous studies and reviews documenting the effects of temperature on the growth performance of fish, but few studies have investigated its effects on protein synthesis (reviewed by McCarthy and Houlihan, 1997; Carter and Houlihan, 2001).

Barramundi, *Lates calcarifer*, is a commercially important farmed species in Australia and Southeast Asia and aquaculture has recently expanded to North America and Europe. Production of these fish within Australia has steadily increased for the past 15 years and this trend is expected to continue (Boonyaratpalin and Williams, 2002). The wild fishery for barramundi in northeastern Australia is a major industry grossing more than 6 million dollars annually and is surpassed by the recreational fishery. Barramundi are endemic across northern Australia and extend north to Southeast Asia and west to the Persian Gulf. Barramundi have a wide thermal tolerance range (15-40°C) and they are commercially cultured in temperatures from 22-35°C, however the high and low extremes temperatures which they are cultured at approach the thermal tolerance for this species (Tucker, *et al.*,

2002). Recent research has focused on determining optimal feeding practices (Williams and Barlow, 1999) and nutritional requirements for juvenile barramundi (Catacutan and Coloso, 1997; Boonyaratpalin, *et al.*, 1998; Coloso, *et al.*, 1999; Williams and Barlow, 1999; Murillo-Gurrea, *et al.*, 2001). However, until recently (Katersky and Carter, 2005) studies have not examined temperatures above 30°C.

The influence of temperature on protein synthesis has been measured in several fish species (reviewed by McCarthy and Houlihan, 1997; Carter and Houlihan, 2001), few studies have concurrently looked at protein synthesis and growth over a temperature range and attempted to relate changes in protein synthesis to the range of temperatures over which growth is optimal. Growth is a reflection of physiological function and therefore protein synthesis might be predicted to also exhibit an asymmetric response to temperature. However, in Atlantic wolfish, *Anarchichas lupis*, white muscle rates of protein synthesis did not display such a pattern and showed a linear response with temperature (McCarthy, *et al.*, 1999). Protein synthesis has only been measured at a limited number of temperatures above the optimal temperature for growth, which makes it difficult to predict the temperature response, and is a component of the present study. With barramundi becoming an increasingly important global aquaculture species, this provides an excellent opportunity to gather data on a fish species which is being cultured in a wide range of temperatures. The aims of the present study were to determine the optimal temperature for feed intake, growth efficiency and protein metabolism in juvenile barramundi.

2.3. MATERIALS AND METHODS

2.3.1. Experimental Diet

A standard diet was formulated according to known dietary requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet was formulated to

contain 50% crude protein and 19.7 MJ kg^{-1} gross energy (Table 1). Fish meal and fish oil were supplied by Skretting (Tasmania, Australia). Vitamins and minerals were supplied by Sigma-Aldrich Pty. Ltd (Sydney, Australia), Vitamin C was supplied as Stay-C from Roche Pharmaceuticals (Roche Vitamins Australia Ltd., Sydney, Australia).

2.3.2. Growth Experiment

Juvenile barramundi (1-2 g) were obtained from WBA Hatcheries (South Australia, Australia). Fish were maintained at the University of Tasmania under constant environmental conditions (salinity: 10 ‰; photoperiod: 24 h light; temperature: 27°C) and stocked into 5 150-l aquariums and maintained at 27°C. Temperatures were adjusted 1°C d^{-1} towards their experimental temperatures of 21, 24, 30 and 33°C, with the exception of the 27°C aquarium that was maintained at a constant temperature. After 6 days all fish were at their experimental temperature. The fish were fed to satiation twice daily for one week at these temperatures. The standard diet was fed to all fish at all times.

At the start of the experiment, 60 fish from each treatment were anesthetized (100 mg L^{-1}, Benzocaine) and individual weight (g) and total length (mm) measured. Fish were randomly separated into 3 18-l tanks. Ten fish were euthanized (400 mg L^{-1}, Benzocaine) and frozen in liquid nitrogen for whole body chemical composition analysis (see below). Water quality was monitored 3 times $week^{-1}$ and water changes done as necessary to keep water quality within the limits for barramundi (Tucker, *et al.*, 2002). Temperature was recorded hourly with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA) and checked manually twice daily.

Fish were fed to satiation twice daily at 0900 and 1800 for 22 days. A pre-weighed ration was provided to each tank and if completely consumed additional pellets were

counted out and provided until feeding ceased. Any uneaten pellets were siphoned out after 10 min and counted in order to determine total daily food consumption. On day 22, fish from 1 replicate from each treatment were not fed for 24 h and then all individual weight (g) and length (mm) were measured. Two fish from each selected tank were used to measure protein synthesis and 5 fish for whole body composition (see below). On day 23, fish from the remaining 2 replicates of each treatment were not fed for 24 hours and sampled as described above.

2.3.3. Protein Synthesis

Rates of protein synthesis were measured following a single injection of ^{3}H-phenylalanine using the flooding-dose method (Garlick, *et al.*, 1980). Twenty-four hours after their last meal, barramundi were aenestitized (100 mg L^{-1}, Benzocaine), weighed and injected with ^{3}H-phynlyalanine into the caudal vein at a concentration of 1 ml 100 g body weight^{-1}. The injection solution contained 150 mmol L-phenylalanine and L-[2,6-^{3}H]phenylalanine (Amersham Pharmacia Biotech, NSW, Australia) in 0.2 µm filtered seawater at pH 7.4. The mean measured specific activity of the injection solution was 1123 ± 118 dpm nmol^{-1} phenylalanine. Following the injection the fish were returned to separate aquaria containing aerated water (10‰) (Houlihan *et al.*, 1986). Incorporation times varied between 60 and 130 min to investigate the time-course of incorporation to ensure elevated free pools concentrations were stable and incorporation was linear.

Following incubation fish were removed from the aquaria, euthanized (400 mg L^{-1}, Benzocaine) and frozen in liquid nitrogen. The subsequent treatment of samples to measure protein-bound and free-pool phenylalanine-specific radioactivities is as described previously (Houlihan *et al.*, 1986, 1990, 1995). The fractional rate of protein synthesis (k_s) was calculated using the equations of Garlick *et al.*, (1980). To calculate whole body protein synthesis (WBk_s) for each fish its white muscle (WM) free pool specific radioactivity was used as an estimate of the whole body free

pool (Carter, *et al.*, 1993). Protein consumption rates (k_c, $\%\cdot d^{-1}$) were calculated based on the final protein content of the fish (g protein consumed·g fish protein^{-1}· day^{-1}, (Houlihan, *et al.*, 1995). Fractional rates of protein growth (k_g, $\%\cdot d^{-1}$) were calculated from the initial and final protein content of fish (Houlihan, *et al.*, 1995) at each temperature (see below Table 3 for chemical composition of fish). Fractional rates of protein degradation (k_d, $\%\cdot d^{-1}$) were determined to be the difference between k_s and k_g (Houlihan, *et al.*, 1995). Protein concentrations were measured using a modification of the folin-phenol method (Lowry, *et al.*, 1951) and RNA concentrations were measured using dual wavelength absorbance (Ashford and Pain, 1985). RNA was also expressed as the capacity for protein synthesis (Cs: mg RNA · g protein^{-1}) and as RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA . d^{-1}) (Sugden and Fuller, 1991).

2.3.4. Chemical Anaylsis

Standard methods were used to determine dry weight (freeze drying to a constant weight); crude protein (Kjeldahl); crude lipid (Bligh and Dyer, 1959); energy (bomb calorimeter); ash by combustion at 550°C for 16h (AOAC, 1995).

2.3.5. Statistical Analysis

Data are presented as mean ± standard error. The normality and homogeneity of data were explored by examining the residual plots. Results were analyzed using a one-way ANOVA (SPSS, version 11.5) and significant results were compared with Tukey's method.

Table 2.1. Ingredient and chemical composition of experimental diet

Ingreadient Compostion ($g \cdot kg^{-1}$)	
Fish meal	730
Fish oil	70
Pre- gelatinized starch	119
CMC	10
Choline chloride	10
Monobasic sodium phosphate (NaH_2PO_4)	10
Vitamin C (Stay-C)	20
Yb_2O_3	1
Vitamin premix[a]	15
Mineral premix[b]	15
Chemical Compostion ($g \cdot kg^{-1}$ DM)	
Dry matter ($g\ kg^{-1}$)	937.0
Crude protein	504.5
Crude lipid	190.5
Ash	128.5
Energy ($MJ \cdot g^{-1}$)	202.5

[a]Vitamin premix ($mg \cdot kg^{-1}$): Vitamin A (7.50), Vitamin D (9.00), Rovimix E50 (150.00), Menadione sodium bisulphate (3.00), Riboflavin (6.00), Calcium D-pantothenate (32.68), Nicontinic Acid (15.00), Vitamin B-12 (0.015), d-biotin (0.23), Folic acid (1.50), Thiamin HCL (1.68), Pyridoxine HCl (5.49), myo-Inositol (450.00), α-cellulose (817.91).

[b]Mineral premix ($mg \cdot kg^{-1}$): $CuSO_4$ $5H_20$ (35.37), $FeSO_4$ $7H_2O$ (544.65), $MnSO_4$ H_2O (92.28), Na_2SeO_3 (0.99), $ZnSO_4$ $7H_2O$ (197.91), KI (2.16), $CoSO_4$ $7H_2O$ (14.31), α-cellulose (612.33).

Table 2.2. Survival, feed intake, growth and growth efficiency (mean ± standard error) of juvenile barramundi at five different temperatures.

Temperature (°C)	21	24	27	30	33
Measured temperature (°C)	20.6±0.51	24.0±0.02	27.3±0.09	30.1±0.02	32.5±0.04
Mean body $weight_{initial}$ (g)	2.44±0.05	2.66±0.07	2.68±0.08	3.44±0.09	3.60±0.08
Mean body $weight_{final}$ (g)	3.27±0.11	4.54±0.18	11.99±0.50	15.32±0.56	16.18±0.71
Survival (%)	100.00 ± 0.00	100.00 ± 0.00	96.67 ± 1.67	98.33 ± 1.67	95.00 ± 5.00
Feed intake ($g \cdot d^{-1}$)	1.25 ± 0.01^{a}	2.05 ± 0.03^{a}	5.62 ± 0.20^{b}	7.50 ± 0.16^{c}	8.66 ± 0.26^{d}
SGR ($\%\cdot d^{-1}$)	1.27 ± 0.07^{a}	2.32 ± 0.12^{b}	6.37 ± 0.12^{c}	6.42 ± 0.05^{c}	6.28 ± 0.41^{c}
FER ($g \cdot g^{-1}$)	0.57 ± 0.05^{a}	0.79 ± 0.05^{a}	1.37 ± 0.02^{b}	1.34 ± 0.05^{b}	1.17 ± 0.09^{b}
PER ($g \cdot g^{-1}$)	1.11 ± 0.09^{a}	1.52 ± 0.09^{a}	2.64 ± 0.03^{b}	2.58 ± 0.09^{b}	2.25 ± 0.17^{b}

Specific Growth Rate (SGR) = [(lnFBW - lnIBW)/d]*100
Feed Efficiency Ratio (FER) = BW gain,g (wet)/Mass of food consumed,g (dry)
Protein Efficiency Ratio (PER) = BW gain (wet)/Mass of Protein fed (dry)
Means with similar or no superscripts ($p<0.05$, $n=3$) were not significantly different between temperatures.

Table 2.3. Body composition (mean ± standard error) of juvenile barramundi at five different temperatures.

Temperature	21	24	27	30	33
Dry matter ($g \cdot kg^{-1}$)	275.7 ± 2.47^{b}	265.8 ± 2.22^{a}	$271.6 \pm 0.68^{a,b}$	$270.1 \pm 1.59^{a,b}$	272.9 ± 1.23^{b}
Crude protein ($g \cdot kg^{-1}$WW)	$158.0 \pm 2.53^{a,b}$	146.0 ± 3.74^{a}	$159.6 \pm 1.41^{a,b}$	$160.2 \pm 4.04^{a,b}$	162.5 ± 1.31^{b}
Crude lipid ($g \cdot kg^{-1}$WW)	61.6 ± 2.05	54.6 ± 3.57	58.3 ± 2.77	56.2 ± 1.43	56.1 ± 1.47
Ash ($g \cdot kg^{-1}$WW)	$39.0 \pm 0.72^{a,b}$	40.3 ± 0.99^{b}	37.6 ± 0.27^{a}	$39.2 \pm 0.42^{a,b}$	37.6 ± 0.32^{a}
Energy ($MJ \cdot kg^{-1}$ WW)	5.77 ± 0.15	5.53 ± 0.06	5.62 ± 0.06	5.79 ± 0.07	5.72 ± 0.03

Initial group (mean ± S.D., n=10): Dry matter, 255.9 ± 8.83 $g \cdot kg^{-1}$, Crude protein, 149.6 ± 1.75 $g \cdot kg^{-1}$ WW, Crude lipid, 42.6 ± 1.14 $g \cdot kg^{-1}$ WW, Ash, 35.6 ± 1.78 $g \cdot kg^{-1}$ WW, Energy, 5.20 ± 0.12 $MJ \cdot kg^{-1}$ WW.
Means with similar or no superscripts ($P<0.05$, n=3) were not significantly different between temperatures.

Table 2.4. Measurements of RNA (mean ± standard error) of juvenile barramundi at five different temperatures.

Temperature	21	24	27	30	33
RNA ($\mu g \cdot mg^{-1}$)	4.28 ± 0.13	4.38 ± 0.12	4.89 ± 0.14	4.32 ± 0.06	4.28 ± 0.24
Cs (mg RNA·g protein^{-1})	19.94 ± 1.81	19.26 ± 1.02	21.08 ± 1.14	17.74 ± 0.79	18.29 ± 0.88
k_{RNA} ($k_s \cdot g^{-1}$ RNA·d^{-1})	2.27 ± 0.54	3.91 ± 0.74	3.14 ± 0.67	3.12 ± 0.21	3.08 ± 0.35

Cs is the RNA to Protein ratio (mg RNA·g protein^{-1}), k_{RNA} is the RNA activity ($k_s \cdot g^{-1}$ RNA·d^{-1}).

2.4. RESULTS

2.4.1. Growth Experiment

Feed intake ($g \cdot d^{-1}$) was significantly higher at 33°C than at 27 and 30°C ($F=233.38$; $df=4,10$; $p<0.001$), which were not different from one another, but were significantly greater than the lower temperature groups (Table 2). At 33°C food consumed was 7.63 ± 0.85 $\%\cdot d^{-1}$, and more than three times that at 21°C. Body weight (g) gain was not significantly different between the 27, 30 and 33°C treatments ($F=71.68$; $df= 4,10$; $p<0.001$), however this was significantly greater than at 21 and 24°C. Specific growth rate (SGR, $\%\cdot d^{-1}$, $F=152.37$; $df= 4,10$; $p<0.001$), growth efficiency (feed efficiency ratio (FER, $F=41.77$; $df=4,10$; $p<0.001$) and protein efficiency ratio (PER, $F=41.77$; $df=4,10$; $p<0.001$) followed the same pattern (Table 2), there were no significant differences among the high (27, 30 and 33°C) or among the low temperature groups (21 and 24°C) and both groups were significantly different from each other.

At 24°C, whole body crude protein was significantly lower than at 33°C but not significantly different to the other temperature groups ($F=3.77$; $df=4,25$; $p=0.016$, Table 3). At 24°C, the ash content was significantly greater than at 27 and 33°C, but no other significant differences occurred ($F=3.68$; $df=4,25$; $p<0.017$). Crude lipid ($F=1.28$; $df= 4,25$; $p=0.303$) and energy content ($F=2.21$; $df= 4,24$; $p=0.098$) were not significantly different among the 5 temperatures (Table 3).

2.4.2. Protein Synthesis

Validation of the flooding dose method was confirmed from the relationship between the *in vivo* incubation times and the bound and free pools of ^{3}H-phenlyalanine ($dpm \cdot nmol^{-1}$ phenylalanine). There was a significant positive linear relationship between the bound ^{3}H-phenylalanine (s_b) and the in vivo incubation time (t)

described by, $S_b = 0.007t+0.267$ ($p= 0.048$; $df = 26$; $r^2 =0.15$). The ^{3}H-phenlyalanine free pool (S_a) significantly decreased over the incubation time (t) according to the equation $S_a = -4.656t + 1534.7$ ($p=0.011$; $df = 26$; $r^2=0.23$) and was corrected for by use of the free pool calculation according to the equation:

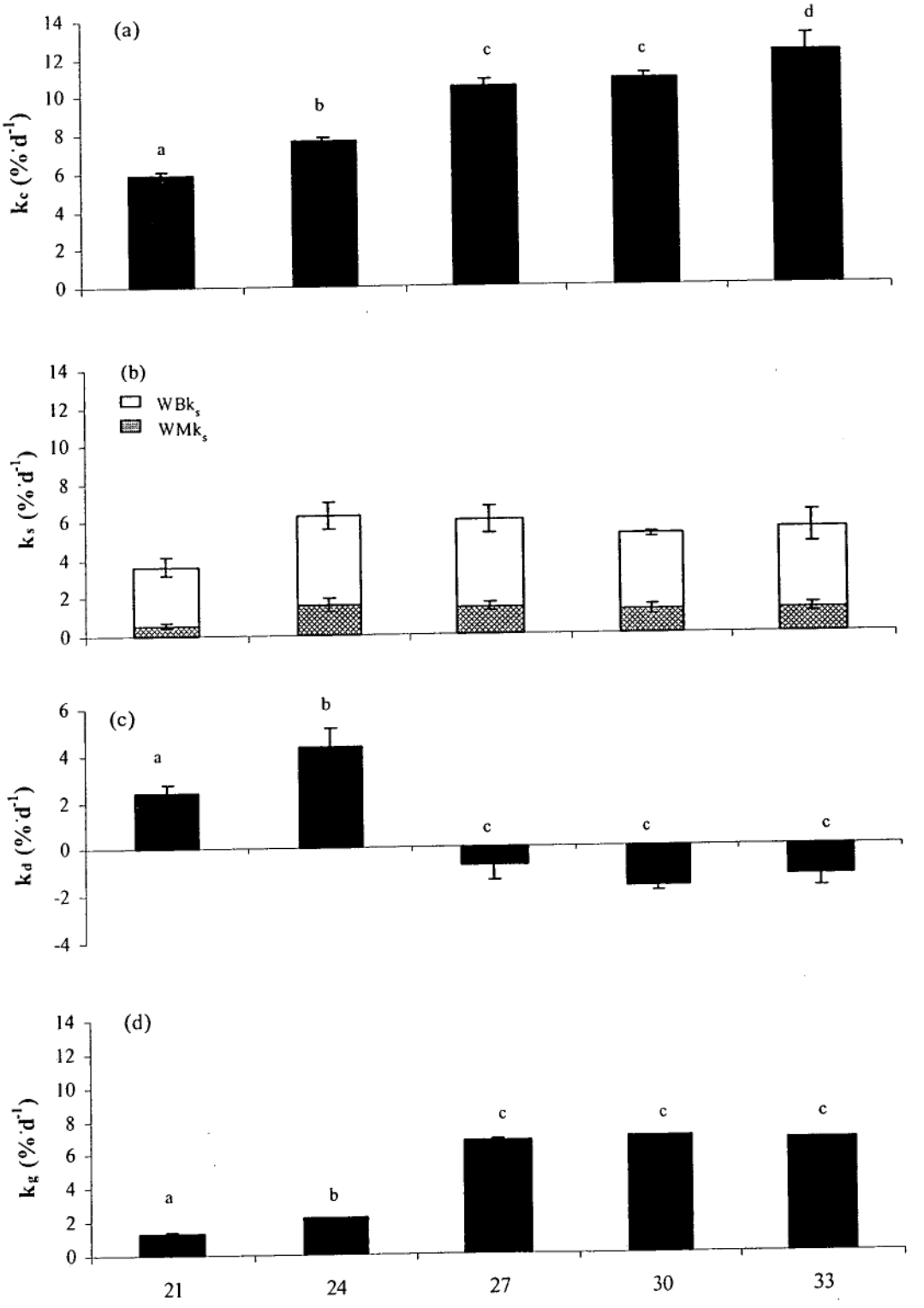
$$k_s (\%\cdot d^{-1}) = (S_{b(t2)} - S_{b(t1)} / S_{a(t2-t1)}) * (1440/t2-t1) * 100 \quad (1)$$

Where k_s is the fractional rate of protein synthesis, $S_{b(t2)}$ is the protein bound specific radioactivity at time 2, $S_{b(t1)}$ is the protein bound specific radioactivity at incorporation time 1, $S_{a(t2-t1)}$ is the mean free pool specific radioactivity at the time period t2-t1 (Houlihan, *et al.*, 1986).

Fractional rate of protein consumption, k_c, increased with increasing temperature with the exception of the 27 and 30°C groups ($F=52.95$; $df=4,9$;$p<0.001$, Fig.1d). No significant differences in k_g existed between 27, 30 and 33°C but were significantly greater than 24 and 21°C ($F=204.59$; $df=4,9$; $p<0.001$, Fig.1b). No significant differences in WBk_s were found between temperatures ($F= 2.83$; $df=4,9$; $p=0.090$, Fig.1a). White muscle k_s at 21°C were significantly lower than at 24 and 27°C ($F=6.20$; $df=4,9$; $p=0.011$). There were no significant differences between 24, 27, 30 and 33°C. A consistent trend appeared for all temperatures with the WBk_s being approximately 4 times that of the WMk_s (Fig. 1a). A significant positive linear relationship existed between WMk_s and WBk_s ($F=0.002$; $df=13$; $r^2=0.57$, Fig. 2). Protein retention efficiency was nearly 50% for the 27, 30 and 33 °C groups, while at low temperatures only 20% of the protein consumed were retained. Fractional rates of protein degradation were significantly different between 21 and 24°C and were different from the degradation rates at 27, 30 and 33°C ($F=20.76$; $df=4,9$; $p<0.001$,Fig 1c). Due to growth rate exceeding the 24 h synthesis rate, fractional rates of protein degradation at 27, 30 and 33°C had negative values.

RNA concentration ($F=2.67$; $df=4,9$; $p=0.102$), Cs ($F=1.06$; $df=4,9$; $p=0.429$) and k_{RNA} ($F=1.07$; $df=4,9$; $p=0.425$) were not significantly different between temperatures and were therefore combined. The mean (± S.D.) RNA concentration, Cs and k_{RNA} were 4.44 ± 0.34 $\mu g \cdot mg^{-1}$, 19.37 ± 2.11 mg RNA·g protein^{-1} and 3.10 ± 0.98 $k_s \cdot g^{-1}$ RNA · d^{-1}, respectively (Table 4). The relationship between Cs and WBk_s was not significant ($p=0.84$; $df= 13$; $r^2=0.004$, Fig. 3a) however, the relationship between k_{RNA} and WBk_s is positive and significant ($p<0.001$; $df=13$; $r^2=0.667$, Fig. 3b) indicating that ribosomal activity and not the concentration of RNA was driving protein synthesis.

Figure 2.1. (a) Protein consumption (k_c, $\%\cdot d^{-1}$), (b) protein synthesis (k_s, $\%\cdot d^{-1}$) for whole body (WBk_s) and white muscle (WMk_s) at 24 h after feeding; (c), protein degradation (k_d, $\%\cdot d^{-1}$) and (d) protein growth (k_g, $\%\cdot d^{-1}$), for juvenile barramundi at five different temperatures.


(a)
k_c (% d^{-1})
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Temperature (°C)

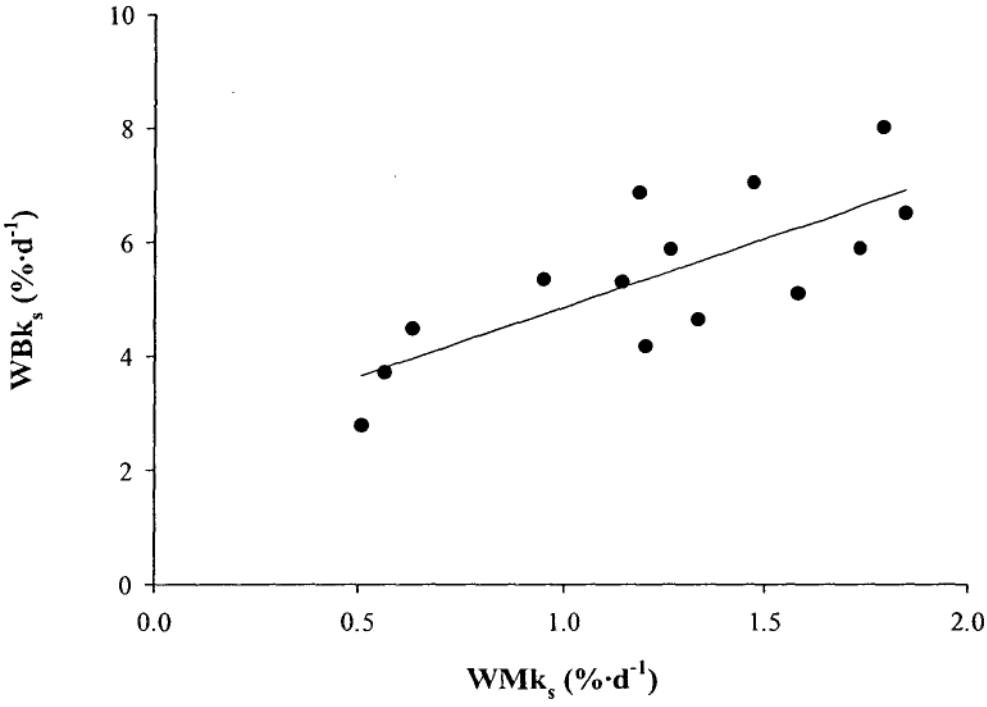
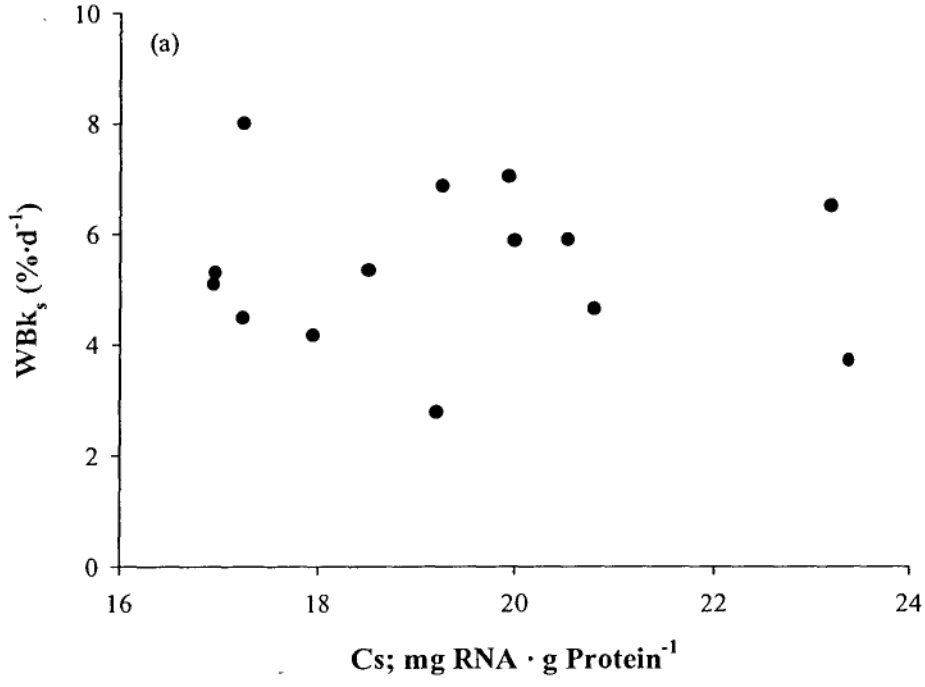


Figure 2.2. The relationship between white muscle rates of protein synthesis (WMk_s, %•d^{-1}) and whole body rates of protein synthesis (WBk_s, %•d^{-1}) for juvenile barramundi, 24 h after feeding, at five different temperatures.

Figure. 2.3. The relationship between (a) Cs (mg RNA · g Protein^{-1}) and the whole body rates of protein synthesis (WBk_s, %·d^{-1}) and (b) RNA activity (k_{RNA}, k_s·g^{-1} RNA· d^{-1}) and WBk_s for juvenile barramundi, at five different temperatures.


(a)
10
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6
4
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0
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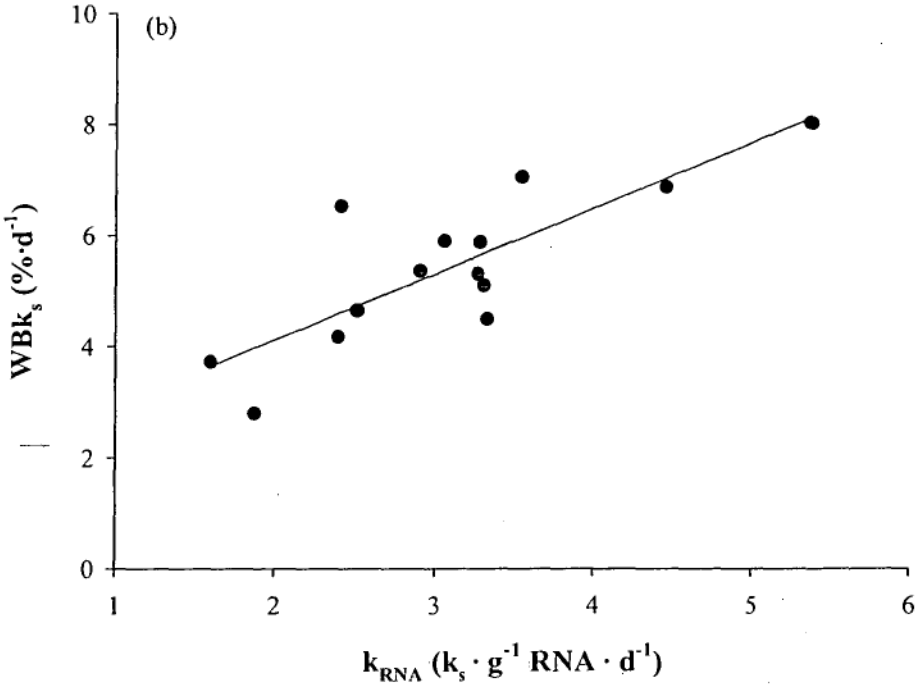
18
20
22
24
WBk$_s$ (%·d^{-1})
Cs; mg RNA · g Protein^{-1}


(b)
10
8
6
4
2
0
1
2
3
4
5
6
WBk$_s$ (%·d^{-1})
k$_{RNA}$ (k$_s$ · g^{-1} RNA · d^{-1})

2.5. DISCUSSION

Although there has been research on the effects of temperature on warm water species (Haschemeyer, *et al.*, 1979; Watt, *et al.*, 1988), the present study is the first to examine feed intake and growth in relation to whole body and tissue protein synthesis in a tropical fish across a wide range of temperatures. It was originally thought that the temperatures selected would include the optimal temperatures for juvenile barramundi, however higher temperatures will have to be examined in order to determine the full extent of the optimal range for feed intake and growth. The results showed that while feed intake, growth and growth efficiency increased overall as temperatures increased, protein synthesis at 24 h after feeding was not different between temperatures of 21-33°C. The selection of a single time point (24 h) can be misleading because it does not take account of post-prandial fluctuations in protein synthesis. This was assumed to be the case in this experiment and therefore the daily rates of protein synthesis have been underestimated at temperatures above 27°C.

2.5.1. Growth Experiment

Although previous research has been done on the feed intake and growth of juvenile barramundi at different temperatures (Williams and Barlow, 1999), however the present study was the first to look at temperatures above 30°C. The findings in the present study are consistent with previous results showing a plateau in FCR between 26 and 29°C (Williams and Barlow, 1999). In the present study this plateau continued to 33°C, since this was the highest temperature tested it made it difficult to determine an optimal temperature for feed intake and growth from this data.

Growth efficiency can remain stable over a wide range of temperatures (Forseth, *et al.*, 2001). The present study showed this to be the case for juvenile barramundi, with high growth efficiency occurring from 27 up to 33°C. Previous research on barramundi has been focused on temperatures between 27 and 30°C (Williams and

Barlow, 1999; Murillo-Gurrea, *et al.*, 2001; Tian and Qin, 2003; Williams, *et al.*, 2003) as this was assumed to be the extent of the optimal range for growth efficiency.

2.5.2. Protein synthesis

2.5.2.1. 24 hour measure

In the present study, differences in protein synthesis in both WM and WB were not significant between the 5 temperatures. This result was surprising due to the large differences in growth between temperatures and consequently resulted in values for k_d which were negative. Clearly, the negative results at temperatures of 27°C and above are not biologically possible and along with there being no difference in synthesis between temperatures argue that daily protein synthesis was underestimated when determined from samples taken at 24 h after feeding. This was attributed to not measuring the post-prandial increase and the daily rate of protein synthesis being determined from measurements which were at pre-feeding levels. The post-prandial protein synthesis has been shown to mimic the SDA and researchers have shown that post-prandial oxygen consumption peaks at approximately twice the pre-feeding rate (reviewed by Jobling, 1981). Therefore it would be possible to estimate the daily rate of protein synthesis from a 24 h measurement if SDA for the species is known (Carter, *et al.*, 1993). Unfortunately, data on the post-prandial oxygen consumption of small barramundi is unknown at this time. The positive values of protein degradation at 21 and 24°C are most likely reflective of more accurate measures of protein synthesis due to the reduced growth and synthesis rates which would be seen at lower temperatures (Jobling, 1994; McCarthy, *et al.*, 1999). These findings are explored further in Chapters 5 and 6. Essentially, protein synthesis exhibits a post-prandial peak (Lyndon, *et al.*, 1992; Chapters 5 and 6) that changes in relation to temperature and increases the daily rate of protein synthesis.

2.5.2.2. WM vs. WB

The relationship between white muscle and whole body protein synthesis was linear regardless of fish species and whole body rates averaged 2-4 times the WM protein synthesis (Houlihan, *et al.*, 1988; Carter and Houlihan, 2001). Strong relationships between WM and WB protein synthesis have now been demonstrated for rainbow trout (Fauconneau, *et al.*, 1990), Atlantic cod (Houlihan, *et al.*, 1988), Atlantic wolfish (McCarthy, *et al.*, 1999) and barramundi (this study) as well as when data from several species were combined (reviewed by Carter and Houlihan, 2001). This relationship holds true irrespective of temperature (McCarthy, *et al.*, 1999). There has been considerable interest in using WM protein synthesis to predict WB protein synthesis and growth rates (Houlihan, *et al.*, 1995). Even though the white muscle has the lowest protein synthesis (and protein turnover rates) of all the major tissues and organs, it has the largest protein mass in fish and is therefore reflective of whole body protein synthesis. In addition, measuring WM protein synthesis is much easier due to smaller samples sizes in comparison to the entire fish (reviewed by Carter and Houlihan, 2001).

2.5.2.3. Mechanisms of protein synthesis

In the present study, variations in protein synthesis were explained by increased k_{RNA} and not differences in RNA concentration or Cs. A number of studies have determined similar relationships and have found that changes in protein synthesis were driven by changes in ribosomal activity (McMillan and Houlihan, 1989; Foster, *et al.*, 1992; Lyndon, *et al.*, 1992). The effect of temperature on mechanisms of protein turnover has also been examined and it has been suggested that the increased concentrations of RNA and Cs at low temperatures are compensatory mechanism which fish adopt to maintain protein synthesis (Foster, *et al.*, 1992; McCarthy, *et al.*, 1999). This does appear to be the case for barramundi as there where no differences

between temperatures and the RNA concentration or Cs. Increased k_{RNA} after feeding and not RNA:protein appear responsible for the regulation of protein synthesis rates (McMillan and Houlihan, 1989; Mathers, *et al.*, 1993).

In conclusion, protein turnover is often determined from long-term growth data (weeks to months), short-term protein synthesis measurements (hours) and a calculation for protein degradation from the two (Carter and Houlihan, 2001). Single measurements of protein synthesis can be misleading by not accounting for the daily fluctuations which occur. In the present study, the single measurement of protein synthesis at 24 h underestimated the daily rate of protein synthesis and this underestimation carried over into the calculation of protein degradation. In chapters 5 and 6 this issue is addressed by examining protein synthesis at various times after feeding at temperatures which span the thermal tolerance range for barramundi (21-39°C) to provide a more accurate estimate of the daily rates of synthesis including the post-prandial increase which occurs at the different temperatures.

2.6. REFERENCES

Association of official Analitical Chemists. 1995. Official methods of analysis. AOAC, Inc. Arlington, VA.

Ashford, A.J., Pain, V.M., 1985. Effects of diabetes in the rates of synthesis and degradation of ribosomes in rat muscle and liver *in vivo*. Journal of Biological Chemistry 261, 4059-4065.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Boonyaratpalin, M., Williams, K., 2002. Asian Sea Bass, *Lates calcarifer*. In: Webster, C.D., C., L. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, pp. 40-50.

Boonyaratpalin, M., Suraneiranat, P., Tunpibal, T., 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. Aquaculture 161, 67-78.

Brett, J.R., Groves, T.D.D., 1979. Physiological Energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Vol. VIII: Bioenergetics and Growth. Acedemic Press, New York, NY, pp. 279-351.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Wright, P., Anderson, P. (Eds.), Fish Physiology, Vol. 20: Nitrogen Excretion. Academic Press, New York, NY, pp. 31-75.

Carter, C.G., Houlihan, D.F., Brechin, J., McCarthy, I.D., 1993. The relationship between protein intake and protein accretion, synthesis and retention efficiency for individual grass carp, *Ctenopharyngodon idella* (Valenciennes). Canadian Journal of Zoology 71, 392-400.

Catacutan, M.R., Coloso, R.M., 1997. Growth of juvenile Asian sea bass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture 149, 137-144.

Coloso, R.M., Murillo-Gurrea, D.P., Borlongan, I.G., Catacutan, M.R., 1999. Sulphur amino acid requirement of juvenile Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology 15, 54-58.

Fauconneau, B., Aguirre, P., Blanc, J.M., 1990. Protein synthesis in different tissues of mature rainbow trout (*Salmo gairdneri* R.). Influence of triploidy. Comparative Biochemistry and Physiology Part C 97, 345-352.

Forseth, T., Hurley, M.A., Jensen, A.J., Elliott, J.M., 2001. Functional models for growth and food consumption of Atlantic salmon parr, *Salmo salar*, from a Norwegian river. Freshwat. Biol. 46, 173-186.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod. Canadian Journal of Zoology 70, 2095-2102.

Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissue by injection of [^{3}H] phenylalanine. Biochemical Journal 192, 719-723.

Haschemeyer, A.E., Persell, R., Smith, M.A.K., 1979. Effect of temperature on protein synthesis in fish of the Galapagos and Perlas Islands. Comparative Biochemistry and Physiology Part B, 91-95.

Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effects of body size. Physiological Zoology 59, 482-493.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995. Protein synthesis in fish. In: Hochachka, P., Mommsen, P. (Eds.), Biochemistry and Molecular Biology of Fishes, pp. 191-220.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45, 951-964.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. Journal of Fish Biology 18, 385-400.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Katersky, R.S., Carter, C.G., 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. Journal of Comparative Physiology B 162, 209-215.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., Burren, L.J., 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. Journal of Fish Biology 43, 245-263.

McCarthy, I., Houlihan, D.F., 1997. The effect of temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (*Salmo salar* L.) stocks in Europe as a result of global warming. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 51-77.

McCarthy, I.D., Moksness, E., Pavlov, D.A., Houlihan, D.F., 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Sciences 56, 231-241.

McMillan, D.N., Houlihan, D.F., 1989. Short-term responses of protein synthesis to re-feeding in rainbow trout. Aquaculture 79, 37-46.

Murillo-Gurrea, D.P., Coloso, R.M., Borlongan, I.G., Serrano, A.E., 2001. Lysine and arginine requirements of juvenile Asian sea bass (*Lates calcarifer*). Journal of Applied Ichthyology 17, 49-53.

Sugden, P.H., Fuller, S.J., 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochemical Journal 273, 21-37.

Tian, X., Qin, J.G., 2003. A single phahse of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. Aquaculture 224, 169-179.

Tucker, J., J.W., , Russel, D.J., Rimmer, M.A., 2002. Barramundi Culture: A success story for aquaculture in Australia, World Aquaculture, pp. 53-59.

Watt, P.W., Marshall, P.A., Heap, S.P., Loughna, P.T., Goldspink, G., 1988. Protein synthesis in tissues of fed and starved carp, acclimated to different temperatures. Fish Physiology and Biochemistry 4, 165- 173.

Williams, K., Barlow, C.G., 1999. Dietary requirements and optimal feeding pratices for barramundi (*Lates calcarifer*). Queensland Department of Primary Industries.

Williams, K., Barlow, C.G., Rodgers, L., Hockings, I., Agcorpra, C., Ruscoe, I., 2003. Asian seabss *Lates calcarifer* perform well when fed pelleted diets high in protein and lipid. Aquaculture 225, 191-206.

CHAPTER 3

GROWTH EFFICIENCY OF JUVENILE BARRAMUNDI, *LATES CALCARIFER* (BLOCH), AT HIGH TEMPERATURES

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3.1. ABSTRACT

Temperature is recognized to be the most important environmental factor affecting growth in fish. Barramundi are cultured over a wide range of temperatures and extreme high temperatures which they can experience under culture approach the upper thermal tolerance limit for this species. A growth trial was conducted on juvenile barramundi to examine the effects of high temperatures ranging from the minimum optimal temperature (27 °C) for growth efficiency to the extreme upper thermal limits (39 °C) for feed intake, growth and growth efficiency. Juveniles (4.87 ± 0.32 g) were held at four different temperatures 27, 33, 36 and 39 °C and fed twice daily to satiation (503.5 g kg^{-1} crude protein, 182.5 g kg^{-1} lipid, 150.1 g kg^{-1} ash, 20.52 GE MJ kg^{-1}). Feed intake ($g \cdot d^{-1}$) and SGR ($\% \cdot d^{-1}$) increased with increasing temperature up to 36° C. At 39 °C feed intake, growth, feed efficiency ratio, protein efficiency ratio and productive energy value were significantly lower than at the other temperatures. This demonstrates that growth was optimized at temperatures from 27 to 36 °C and that barramundi have a much wider range for maximum growth efficiency than preciously thought.

Keywords: Barramundi, Asian Sea Bass, Temperature, Growth efficiency

3.2. INTRODUCTION

Temperature has a marked effect on many key physiological processes in fish (Brett and Groves, 1979) and there have been numerous studies (Jobling, 1981; McCarthy, et al., 1998; Jonassen, et al., 1999) and reviews (Elliott, 1982) which have examined the effects of temperature on growth. Every species has a range of temperatures over which it survives and where growth occurs. However, growth is maximized at an optimal temperature within this thermal tolerance range (Jobling, 1997). As temperature increases, feed intake will increase to a maximum and then decrease rapidly prior to the upper limit for thermal tolerance (Jobling, 1994). Maximum feed intake will occur at a temperature which is generally a few degrees above the optimal temperature for growth. Metabolic rate increases exponentially as the temperature increases and, at any given temperature, the difference between feed intake and metabolic rate will determine the energy available for growth (Brett and Groves, 1979; Jobling, 1994). For cultured species, these parameters are extremely important in order to understand and maximize the efficiency with which the consumed food is converted into growth (Jobling, 1994; Carter, et al., 2001).

Barramundi or Asian sea bass, *Lates calcarifer*, is a commercially important aquaculture species in Australia and southeast Asia. Production of these fish in Australia has steadily increased for the past 15 years and this trend is expected to continue (Boonyaratpalin and Williams, 2002). Barramundi have an extremely wide thermal tolerance range (15-40°C) and they are cultured in temperatures from 22-35°C (Tucker et al., 2002). However, the more extreme temperatures under which they are cultured approach the thermal tolerance for this species. Recent research has focused on determining optimal feeding practices (Williams and Barlow, 1999) and nutritional requirements for juvenile barramundi (Catacutan and Coloso, 1997; Boonyaratpalin, et al., 1998; Coloso et al., 1999; Williams and Barlow, 1999; Murillo-Gurrea et al., 2001). However, studies have not examined temperatures above 30°C. Previous research has shown that growth efficiency had an optimal range of 27 to 33°C (Chapter 2). However, the highest temperature examined was

33°C and the full temperature range for maximum growth efficiency was not determined. This study examines feed intake and growth efficiency at temperatures which range from the minimum optimal temperature (27°C) for growth efficiency to the extreme upper thermal limits (39°C) for juvenile barramundi in order to determine the entire range of temperatures where growth is optimized.

3.3. MATERIALS AND METHODS

3.3.1. Experimental Diet

A standard diet (2-mm pellets) was formulated according to known dietary requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet was formulated to contain 50% crude protein and 19.7 MJ kg^{-1} gross energy (Table 1). Fish meal and fish oil were supplied by Skretting (Cambridge, TAS, Australia). Vitamins and minerals were supplied by Sigma-Aldrich Pty. Ltd (Sydney, NSW, Australia), Vitamin C was supplied as Stay-C (Roche Vitamins Australia Ltd., Sydney, NSW, Australia).

3.3.2. Growth Experiment

Juvenile barramundi, *Lates calcarifer,* (1 g) were obtained from WBA Hatcheries (West Beach, SA, Australia). Fish were maintained at the University of Tasmania under constant environmental conditions (salinity: 10 ‰; photoperiod: 24h light (Barlow et al., 1995); temperature: 27.0°C). Fish were initially stocked into 4 150-l aquaria and maintained at 27.0°C. Temperature was adjusted 1°C d^{-1} towards the experimental temperatures of 33.0, 36.0 and 39.0°C, with the exception of the 27.0°C aquarium which was maintained at a constant temperature. After 12 days all fish were at their experimental temperature. The experiment was conducted in four identical recirculating systems each consisting of 3 19-l carboys with a trickle biofilters on each system. Each system was held at a constant temperature (either 27, 33, 36 or 39°C) with submersible heaters each controlled with an individual

thermostat. The system was modified from that used by (Engin and Carter, 2001). The fish were fed to satiation twice daily during this acclimation period. The standard diet was fed to all fish at all times (Table 1).

At the start of the experiment, 60 fish from each treatment were anesthetized (100 mg l^{-1}, benzocaine) and individual weight (g) was measured. These fish were then randomly separated into 3 19-l recirculating tanks to give 20 fish per experimental tank. Ten fish were killed with an overdose of benzocaine (400 mg l^{-1}) and frozen in liquid nitrogen for whole-body chemical analysis. Water quality was monitored 3 times $week^{-1}$ and water changes (~50%, with preheated water) were performed as necessary to keep water quality within the limits for barramundi (Tucker et al., 2002). Dissolved oxygen was maintained at >80 % saturation with constant aeration in all temperature treatments. Temperature was recorded twice daily with a mercury thermometer.

Fish were fed to satiation twice daily at 0900 and 1800 for 20 d. A pre-weighed ration was provided to each tank, and if completely consumed additional pellets were counted out and provided until feeding ceased. Any uneaten pellets were siphoned out after 10 min and counted in order to determine total daily food consumption. On day 20, fish from 1 replicate from each of the four temperature treatments were starved for 24 h. Following this starvation period, individual weight (g) and total length (mm) were measured for all fish. Five fish from each tank were killed with an overdose of benzocaine (400 mg l^{-1}), autoclaved and freeze-dried to a constant weight in order to determine whole-body chemical composition. On days 21 and 22 fish from the remaining 2 replicates of each treatment were starved for 24 h and sampled as described above.

3.3 Calculations

The following equations were used to calculate specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), productive protein value (PPV) and productive energy value (PEV):

$$SGR\ (\%\cdot d^{-1}) = [(\ln FBW - \ln IBW)/d]*100 \quad (1)$$

$$FER\ (\%) = \text{BW gain, g (wet)} / \text{Mass of food consumed, g (dry)} \quad (2)$$

$$PER\ (\%) = \text{BW gain (wet)} / \text{Mass of protein fed (dry)} \quad (3)$$

$$PPV = (\text{fish protein gain (g CP)}/\text{total protein composition (g CP)})]*100 \quad (4)$$

$$PEV = (\text{fish energy gain (g MJ)}/ \text{ total energy consumed (g MJ)})*100 \quad (5)$$

3.3.3. Chemical Anaylsis

Standard methods were used to determine dry weight (freeze drying to a constant weight); crude protein (Kjeldahl); crude lipid (Bligh and Dyer, 1959); energy (bomb calorimeter); ash by combustion at 550°C for 16 h (AOAC, 1995).

3.3.4. Statistical Analysis

Data are presented as mean ± standard error. The normality and homogeneity of data were explored by examining the residual plots. Results were analyzed using a one-way ANOVA (SPSS, version 11.5) and significant results were compared using Tukey's HSD. Growth data were analyzed using ANCOVA (SPSS, version 11.5) between the initial and final weight measurements as significant differences in mean weights of fish were found between treatments at the start of the experiment.

Table 3.1. Ingredient and chemical composition of experimental diet.

Ingreadient compostion (g kg^{-1})	
Fish meal	730
Fish oil	70
Starch	119
CMC	10
Choline chloride	10
Phosphorus ($NaPO_4$)	10
Vitamin C (Stay-C)	20
Ytterbium-oxide	1
Vitamin premix[a]	15
Mineral premix[b]	15
Chemical compostion (g kg^{-1} DM)	
Dry matter (g kg^{-1})	946.9
Crude protein	503.5
Crude lipid	182.5
Ash	150.1
Energy (MJ kg^{-1})	20.52

[a]Vitamin premix (mg kg^{-1}): Vitamin A (7.50), Vitamin D (9.00), Rovimix E50 (150.00), Menadione sodium bisulphate (3.00), Riboflavin (6.00), Calcium D-pantothenate (32.68), Nicontinic Acid (15.00), Vitamin B-12 (0.015), d-biotin (0.23), Folic acid (1.50), Thiamin HCL (1.68), Pyridoxine HCl (5.49), myo-Inositol (450.00), α-cellulose (817.91).

[b]Mineral premix (mg kg^{-1}): $CuSO_4$ $5H_20$ (35.37), $FeSO_4$ $7H_2O$ (544.65), $MnSO_4$ H_2O (92.28), Na_2SeO_3 (0.99), $ZnSO_4$ $7H_2O$ (197.91), KI (2.16), $CoSO_4$ $7H_2O$ (14.31), α-cellulose (612.33).

3.4. RESULTS

Feed intake ($g \cdot d^{-1}$) was significantly higher at 33 and 36°C than at 27 and 39°C ($F=29.40$; $df=3,8$; $P<0.001$), which were not significantly different from one another (Table 2). At 33 and 36°C feed intake was 10.48 ± 0.50 and 9.74 ± 0.99 $g \cdot d^{-1}$, respectively. This was approximately double the intake at 27 and 39°C. Significant differences found among initial weights ($F=21.64$; $df= 3,236$; $P<0.001$) did not have a significant effect on final weights ($F=1.31$; $df= 3,225$; $P=0.253$). Body weight gain (g) ($F=63.40$; $df=3,8$; $P<0.001$) and specific growth rate (SGR)($F=72.38$; $df=3,8$; $P<0.001$) were both significantly higher at 33 and 36°C than at 27 and 39°C. Fish reared at 39 °C had significantly lower growth than at the other temperatures. Overall, as temperature increased there was an increase in both feed intake and SGR up to 36°C. At 39°C a sharp decrease in feed intake and growth was observed (Table 2).

Whole-body crude protein was significantly ($F=9.35$; $df=3,20$; $P<0.001$) lower in the 39°C treatment than the other temperatures which were not significantly different from one another. Crude lipid was also significantly ($F=4.70$; $df= 3,22$; $P=0.011$) lower at 39°C than at 33 and 27°C but not different from the 36°C treatment. Ash content at 39°C was significantly ($F=8.03$; $df=3,20$; $P=0.001$) higher than at the 33 and 27°C groups but not different from the 36°C. Energy did not differ among the temperature treatments ($F=2.89$; $df=3,20$; $P=0.061$), (Table 3).

Growth efficiency expressed as feed efficiency ratio (FER), protein efficiency ratio (PER) and productive energy value (PEV) were significantly lower for fish held at 39°C, than the remaining treatments (FER, $F=68.72$; $df=3,8$; $P<0.001$: PER, $F=68.25$; $df=3,8$; $P<0.001$, Table 2: PEV, $F=84.23$; $df=3,8$; $P<0.001$, Fig. 1). Productive protein value (PPV) was also significantly lower for fish held at 39°C than the remaining treatments, however the 27°C was significantly lower than the 33°C treatment ($F=148.36$; $df=3,8$; $P<0.001$, Fig. 1).

Table 3.2. Survival, feed intake, growth and growth efficiency (mean ± standard error) of juvenile barramundi at four different temperatures.

Temperature (°C)	27	33	36	39
Measured temperature (°C)	26.98 ± 0.04	33.16 ± 0.13	35.36 ± 0.32	38.86 ± 0.05
Mean body weight$_{initial}$ (g)	4.23 ± 0.13	5.06 ± 0.19	4.49 ± 0.12	5.69 ± 0.17
Mean body weight$_{final}$ (g)	13.11^{b} ± 0.45	22.47^{a} ± 1.71	20.09^{a} ± 1.76	10.33^{c} ± 0.14
Survival (%)	98.33 ± 1.67	96.67 ± 1.67	98.33 ± 1.67	95.00 ± 2.89
Feed intake($g \cdot d^{-1}$)	5.58^{b} ± 0.14	10.48^{a} ± 0.50	9.74^{a} ± 0.99	4.32^{b} ± 0.08
SGR ($\% \cdot d^{-1}$)	5.38^{b} ± 0.19	7.10^{a} ± 0.07	7.11^{a} ± 0.42	2.84^{c} ± 0.09
FER ($g \cdot g^{-1}$)	1.41^{a} ± 0.05	1.46^{a} ± 0.01	1.46^{a} ± 0.02	0.86^{b} ± 0.04
PER ($g \cdot g^{-1}$)	2.80^{a} ± 0.09	2.91^{a} ± 0.02	2.82^{a} ± 0.05	1.72^{b} ± 0.08

Means with similar superscripts were not significantly different ($P<0.05$, $n=3$).

Table 3.3. Body composition (mean ± standard error) of juvenile barramundi at four temperatures.

Temperature (°C)	27	33	36	39
Dry Matter ($g \cdot kg^{-1}$)	$262.5^{b} \pm 0.17$	$275.8^{a} \pm 0.14$	$275.6^{a} \pm 0.08$	$263.0^{b} \pm 0.23$
Crude Protein ($g \cdot kg^{-1}$WW)	$154.9^{b} \pm 1.01$	$164.2^{a} \pm 1.25$	$163.8^{a} \pm 1.54$	$147.4^{c} \pm 1.29$
Crude Lipid ($g \cdot kg^{-1}$WW)	$63.5^{a} \pm 1.27$	$63.1^{a,b} \pm 2.42$	$64.4^{a} \pm 2.96$	$54.0^{b} \pm 2.39$
Ash ($g \cdot kg^{-1}$WW)	$37.8^{b} \pm 0.37$	$39.6^{b} \pm 0.78$	$42.1^{a,b} \pm 1.31$	$44.2^{a} \pm 1.53$
Energy ($MJ \cdot kg^{-1}$ WW)	$5.76^{a,b} \pm 0.07$	$5.96^{a} \pm 0.09$	$6.09^{a} \pm 0.08$	$5.48^{b} \pm 0.09$

Initial group (mean ± SD: n=10): Dry matter, 245.3 $g \cdot kg^{-1}$, Crude protein, 153.3 ± 0.54 $g \cdot kg^{-1}$ WW, Total lipid, 56.0 ± 3.19 $g \cdot kg^{-1}$ WW, Ash, 34.1 ± 1.20 $g \cdot kg^{-1}$WW, Energy, 5.14 ± 0.01 $MJ \cdot kg^{-1}$ WW. Means with similar superscripts ($P<0.05$, n=3) were not significantly different between temperatures.

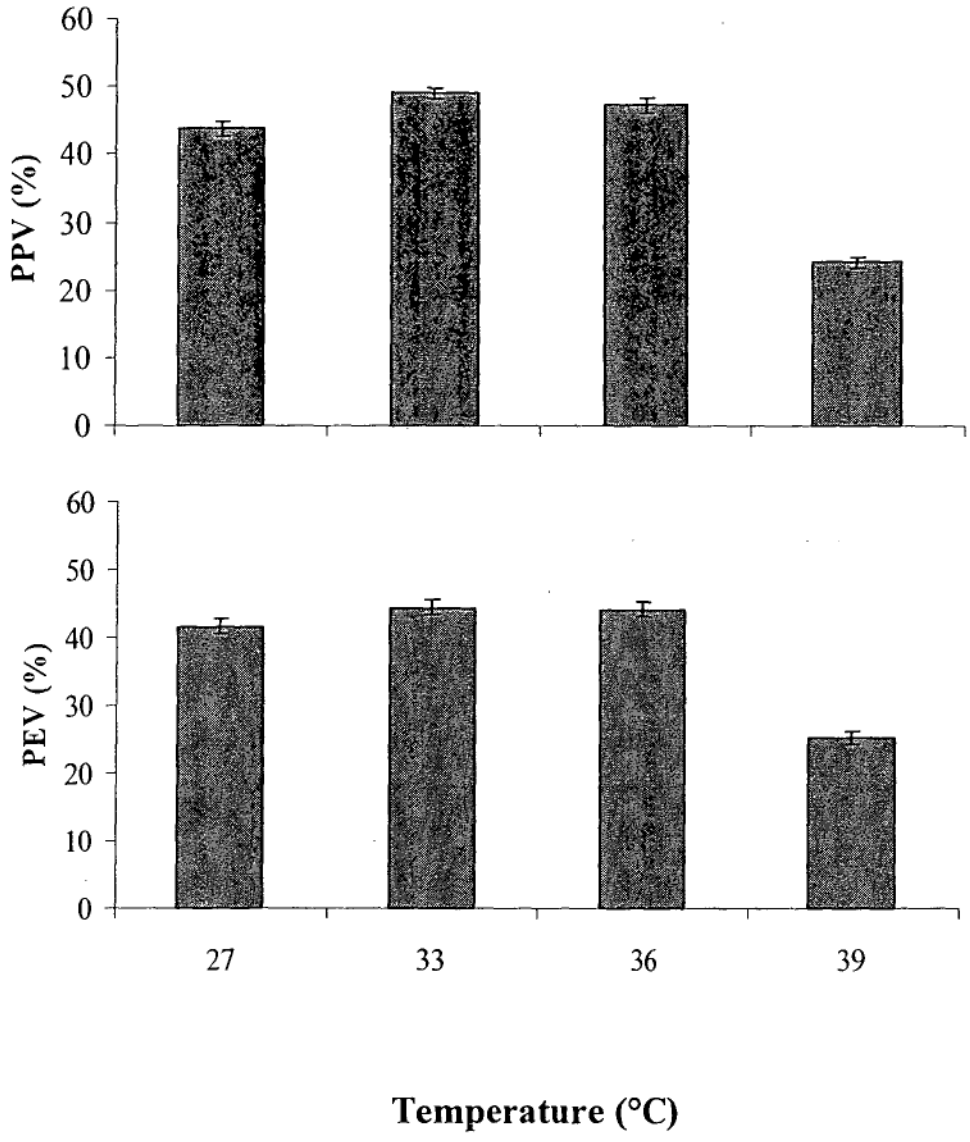


Figure 3.1. Growth efficiency (mean ± standard error) of juvenile barramundi expressed as productive protein value (PPV %) and productive energy value (PEV %). Means with similar letters were not significantly different ($p<0.05$, $n=3$).

3.5. DISCUSSION

The present study was the first to examine feed intake, growth and growth efficiency in juvenile barramundi over a wide range of high temperatures. The results show that feed intake and SGR were highest at 33 and 36°C. However, when growth efficiency (PEV, PER and FER) was examined there was no significant difference from 27 to 36°C. It was only when temperatures exceeded 36°C that a decline in efficiency was observed.

Previous research on the feed intake and growth of juvenile barramundi at different temperatures (Williams and Barlow, 1999) examined temperatures ranging from 20 to 29°C and observed a plateau in food conversion ratio (FCR) and feed intake from 26°C to 29°C. This is consistent with previous results (Katersky and Carter, unpublished data), where feed intake (%BW·d^{-1}) remained constant between 27 and 30°C but there was a significant increase between 30 and 33°C. In the present study, feed intake was significantly higher at 33°C than at 27°C.

There is an inverse relationship between SGR and fish weight, SGR decreases as fish weight increases (Jobling, 1994). The majority of research on barramundi has been on larger slower growing juvenile fish, this partly explains the higher SGR's found in the present study. Williams and Barlow (1999) determined SGR of different size barramundi at 29°C and found SGR decreased from 3.6 %·d^{-1} for 40 g fish, through 1.7 for 100g fish and 1.5 for 170g fish to 1.1 for 270g fish. A few studies have looked at growth rates of small juvenile barramundi, Eusebio and Coloso (2000) determined SGR to be 4.1 %·d^{-1} for 40g fish raised at 27-28°C and Catacutan and Coloso (1997) found SGR of 5.0 %·d^{-1} when water temperatures ranged from 26.5 to 29°C for 57 g fish. In the present study, using 5 g fish, SGR was 5.6 %·d^{-1} at 27°C and increased to 7.1 %·d^{-1} at 33 and 36°C.

Temperature has been shown to have an effect on the biochemical composition of fish (Cui and Wootton, 1988; Koskela et al., 1997; Bendiksen et al., 2003; Tidwell et

al., 2003). In the present study, as temperatures exceeded the optimal range for growth efficiency there was a significant decrease in whole-body protein. This differs from Baltic salmon reared at high temperatures where protein did not change with increasing temperature (Koskela et al., 1997). However, whole-body lipid levels peaked at optimal growth temperatures for Baltic salmon (Koskela et al., 1997) and then significantly decreased as the temperature continued to increase. In the present study, lipid levels remained elevated up to 36°C and then significantly declined. The decrease in both protein and lipid at the high temperatures can be attributed to the increased metabolism which is encountered at temperatures nearing the upper limits of thermal tolerance (Jobling, 1997).

Growth efficiency has been shown to remain constant over a wide range of temperatures (Forseth et al., 2001). The present study showed this to be the case for juvenile barramundi, with maximum growth efficiency occurring between 27 and 36°C. As temperatures approached the upper limit for thermal tolerance (39°C), growth efficiency declined due to an increase in metabolism and a decrease in feed intake (Jobling, 1997). Previous research on barramundi has been focused on temperatures between 27 and 30°C (Williams and Barlow, 1999; Murillo-Gurrea et al., 2001; Tian and Qin, 2003; Williams et al., 2003) as this was believed to be the extent of the optimal range for growth efficiency, however, this research shows that juvenile barramundi have a much wider optimal range for culture than previously believed. This is possibly a biological adaptation that barramundi have developed based on their wide geographical distribution around northern Australia and southeast Asia. Barramundi are an incredibly robust fish thus allowing them to grow and thrive in a wide range of environmental conditions.

3.6. REFERENCES

Association of official Analytical Chemists. 1995. Official methods of analysis. AOAC, Inc. Arlington, VA.

Bendiksen, E.A., Berg, A., Jobling, M., Arnesen, A.M., Masoval, K., 2003. Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. Aquaculture 224, 283-299.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Boonyaratpalin, M., Williams, K., 2002. Asian Sea Bass, *Lates calcarifer*. In: Webster, C.D., C., L. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, pp. 40-50.

Boonyaratpalin, M., Suraneiranat, P., Tunpibal, T., 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. Aquaculture 161, 67-78.

Brett, J.R., Groves, T.D.D., 1979. Physiological Energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Vol. VIII: Bioenergetics and Growth. Acedemic Press, New York, NY, pp. 279-351.

Carter, C.G., Houlihan, D.F., Kiessling, A., Medale, F., Jobling, M., 2001. Physiological effects of feeding. In: Houlihan, D.F., Boujard, T., Jobling, M. (Eds.), Food Intake in Fishes. Blackwell Scientific, Oxford, pp. 297-331.

Catacutan, M.R., Coloso, R.M., 1997. Growth of juvenile Asian sea bass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture 149, 137-144.

Coloso, R.M., Murillo-Gurrea, D.P., Borlongan, I.G., Catacutan, M.R., 1999. Sulphur amino acid requirement of juvenile Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology 15, 54-58.

Cui, Y., Wootton, R.J., 1988. Effects of ration, temperature and body size on the body composition and energy content of the minnow, *Phoxinus phoxinus* (L.). Journal of Fish Biology 32, 749-764.

Elliott, J.M., 1982. The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comparative Biochemistry and Physiology Part B 73B, 81-91.

Engin, K., Carter, C.G., 2001. Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary

protein level. Aquaculture 194, 123-136.

Eusebio, P.S., Coloso, R.M., 2000. Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology. 16, 56-60.

Forseth, T., Hurley, M.A., Jensen, A.J., Elliott, J.M., 2001. Functional models for growth and food consumption of Atlantic salmon parr, *Salmo salar*, from a Norwegian river. Freshwater Biology 46, 173-186.

Jobling, M., 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. Journal of Fish Biology 18, 87-96.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Jonassen, T.M., Imsland, A.K., Stefansson, S.O., 1999. The interaction of temperature and fish size on growth of juvenile halibut. Journal of Fish Biology 54, 556-572.

Koskela, J., Pirhonen, J., Jobling, M., 1997. Feed intake, growth rate and body composition of juvenile Baltic salmon exposed to different constant temperatures. Aquaculture International. 5, 351-360.

McCarthy, I., Moksness, E., Pavlov, D.A., 1998. The effects of temperature on growth rate and growth efficiency of juvenile common wolffish. Aquaculture International. 6, 207-218.

Murillo-Gurrea, D.P., Coloso, R.M., Borlongan, I.G., Serrano, A.E., 2001. Lysine and arginine requirements of juvenile Asian sea bass (*Lates calcarifer*). Journal of Applied Ichthyology. 17, 49-53.

Tian, X., Qin, J.G., 2003. A single phahse of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. Aquaculture 224, 169-179.

Tidwell, J.H., Coyle, S.D., Bright, L.A., VanArnum, A., Yasharian, D., 2003. Effects of water temperature on growth, survival, and biochemical composition of largemouth bass *Micropterus salmonides*. Journal of the World Aquaculture Society 34, 175-183.

Tucker, J., J.W., , Russel, D.J., Rimmer, M.A., 2002. Barramundi Culture: A success

story for aquaculture in Australia, World Aquaculture, September. pp. 53-59.

Williams, K., Barlow, C.G., 1999. Dietary requirements and optimal feeding pratices for barramundi (*Lates calcarifer*). Queensland Department of Primary Industries. Queensland, Australia.

Williams, K., Barlow, C.G., Rodgers, L., Hockings, I., Agcorpra, C., Ruscoe, I., 2003. Asian seabss *Lates calcarifer* perform well when fed pelleted diets high in protein and lipid. Aquaculture 225, 191-206.

CHAPTER 4

MODELLING THE EFFECT OF TEMPERAUTRE ON FEED INTAKE AND GROWTH EFFICIENCY OF JUVENILE BARRAMUNDI, *LATES CALCARIFER*

4.1. ABSTRACT

Temperature has a marked and direct effect on many of the key physiological processes in ectotherms, including most fish. Feed intake and growth performance respond asymmetrically to temperature and modelling these relationships provides important information on how fish alter their metabolism at different temperatures. Data from two growth trials on juvenile barramundi (~3-5 g) are presented to model the effects of temperature on the feed intake and growth performance, measured as specific growth rate (SGR), productive protein value (PPV) and productive energy value (PEV), over the thermal tolerance of the species (21-39 °C, at 3 °C intervals). No significant relationships were found between the chemical composition of the fish and temperature. Optimal temperatures were determined from quadratic polynomials: maximum feed intake ($g \cdot d^{-1}$), maximum growth (SGR, $\% \cdot d^{-1}$) and growth efficiency (PPV and PEV, %) occurred at 31 °C, however the magnitude of the range of temperatures where growth efficiency remained maximum was above ten times greater than for feed intake or SGR. These models also found optimal growth to be approximately 4°C higher then previously determined.

Keywords: Barramundi; feed intake; growth efficiency; *Lates calcarifer;* temperature

4.2. INTRODUCTION

Temperature has a marked and direct effect on many of the key physiological processes in ectotherms including fish (Brett and Groves, 1979) on which there have been numerous studies (Jobling, 1981; McCarthy, *et al.*, 1998; Jonassen, *et al.*, 1999) and reviews (Elliott, 1982; Houlihan, 1991; Jobling, 1997). Each species has a range of temperature over which it survives, the thermal tolerance range, and a narrower range where growth occurs. As temperature increases within the thermal tolerance range feed intake increases to a maximum and then decreases rapidly prior to the upper limit for thermal tolerance (Jobling, 1994), while simultaneously the metabolic rate increases exponentially. Therefore, at high temperatures metabolic demands increasingly account for a larger proportion of the ingested energy until growth is compromised (Brett and Groves, 1979; Jobling, 1994). The optimal temperature for growth reflects the temperature where the difference between the ingested energy and the energy expenditures is largest and energy partitioned into growth is maximal.

Barramundi, *Lates calcarifer*, provides an excellent model species to examine the effects of temperature on feed intake and growth efficiency because it has a wide thermal tolerance range (15-40°C). It is also a commercially important fisheries and aquaculture species around the world. As the culture of barramundi expands to geographic locations outside its natural range, it is often conducted at extreme temperatures which approach the lower and upper thermal tolerance limits of the species. Interestingly, this situation is similar to that of Atlantic salmon, *Salmo salar*, which have a temperature tolerance of -0.5-25°C (Wallace, 1993) and are farmed under widely different temperature regimes. It is common in the salmon industry for fish to be exposed to low temperatures, <5 °C, in the northern hemisphere (Koskela, *et al.*, 1997) and extreme high temperatures 20-22°C in the southern hemisphere (Roberts, *et al.*, 2001). Models for feed intake, growth and growth efficiency over the thermal tolerance range for salmon have shown that growth efficiency is maintained

at a optimal level over a much wider temperature range than either feed intake or growth rate (Forseth, *et al.*, 2001). Growth efficiency in barramundi is also maintained at high levels over a wide temperature range (27-36°C, Katersky and Carter, 2005). In order to maintain high growth efficiency the protein and energy requirements in the diet have to meet the species requirement (reviewed by Wilson, 2002). Dietary requirements have been shown to change with temperature (reviewed by Wilson, 2002) therefore it is apparent that when growth efficiency plateaus over a wide range of temperatures, the nutrient requirements would also need to be on a stable plane in order for high efficiency to occur.

The aim of the current paper is to present models for feed intake, growth and growth efficiency, expressed in relation to protein and energy retention, for juvenile barramundi at temperatures which span the thermal tolerance range for the species (21-39°C). Two experiments were conducted. Chapter 2 examined temperatures from 21 to 33 °C (in 3°C increments) and Chapter 3 examined temperatures 27, 33, 36 and 39 °C (Katersky and Carter, 2005). By combining these experiments it was possible to determine optimal temperatures and temperature ranges for maximum feed intake, growth and growth efficiency.

4.3. MATERIALS AND METHODS

4.3.1. Experimental Diet

A standard diet was formulated according to known dietary requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet was formulated to contain 50% crude protein (CP) and 19.7 MJ kg^{-1} gross energy (GE) and as 24 .5 g CP·MJ GE^{-1} (Katersky and Carter, 2005). Fish meal and fish oil were supplied by Skretting (Tasmania, Australia). Vitamins and minerals were supplied by Sigma-Aldrich Pty. Ltd (Sydney, Australia), Vitamin C was supplied as Stay-C from Roche Pharmaceuticals (Roche Vitamins Australia Ltd., Sydney, Australia).

4.3.2. Growth Experiment

The following details applied to both experiments. Stock juvenile barramundi (1 g) were obtained from WBA Hatcheries (South Australia, Australia). Fish were maintained at the University of Tasmania under constant environmental conditions (salinity: 10 ‰; photoperiod: 24h light; temperature: 27°C) in 150-L aquaria. At the start of each experiment, 60 fish from each temperature treatment were anesthetized (100 mg L^{-1}, benzocaine) and individual weights (g) were measured (Table 4.1). Fish were randomly separated into 3 18-L tanks. Water quality was monitored 3 times $week^{-1}$ and water changes were done as necessary to keep water quality within the limits for barramundi (Tucker, *et al.*, 2002). The experiments were conducted in identical recirculating systems each consisting of 3 19-L carboys with a trickle biofilter on each system (Katersky and Carter, 2005). Each system was held at a constant temperature. Fish were fed to satiation twice daily during the acclimation period. The standard diet was fed to all fish at all times and during the experimental period, fish were fed to satiation twice daily at 0900 and 1800. A pre-weighed ration was provided to each tank and if completely consumed additional pellets were counted out and provided until feeding ceased. Any uneaten pellets are siphoned out after 10 minutes and counted in order to determine total daily feed intake. The following equations were used to calculate specific growth rate (SGR), productive protein value (PPV) and productive energy value (PEV):

$$SGR\ (\%\cdot d^{-1}) = [(\ln BW_F - \ln BW_I)/d]*100 \quad (1)$$

$$PPV\ (\%) = (\text{fish protein gain (g CP)/total protein consumed (g CP)})*100 \quad (2)$$

$$PEV\ (\%) = (\text{fish energy gain (g MJ)/ total energy consumed (g MJ)})*100 \quad (3)$$

At the end of the growth experiments, five fish were killed with an overdose of Benzocaine (400 mg L^{-1}) from each replicate tank, autoclaved and freeze-dried to a constant weight in order to determine whole-body chemical composition (Table 4.2).

4.3.2.1. Experiment 1

These data were obtained from the experiment described in Chapter 2. Fish (2.96 ± 0.13 g) were stocked into 5 150-L aquaria and maintained at 27°C. Temperatures were adjusted 1°C d^{-1} towards there experimental temperature 21, 24, 30 and 33°C, with the exception of 27°C aquarium which was maintained at a constant temperature. After 6 days all fish were at their experimental temperature. Temperature was recorded hourly with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA) and checked manually twice daily (Table 4.1). On day 22, fish from one replicate from each of the four treatments were starved for 24 h. Following this starvation period, individual weights (g) and lengths (mm) were measured for all fish. On day 23, fish from the remaining 2 replicates of each treatment were starved for 24 h and sampled as described above.

4.3.2.2. Experiment 2

These data were obtained from the experiment described in Chapter 3. Fish (4.87 ± 0.18 g) were stocked into 4 150-L aquaria and maintained at 27°C. Temperature was adjusted 1°C d^{-1} towards the experimental temperatures of 33, 36 and 39°C, with the exception of the 27°C aquarium which was maintained at a constant temperature. After 12 days all fish were at their experimental temperature. Temperature was recorded twice daily with a mercury thermometer (Table 4.1). On day 20, fish from 1 replicate from each of the four temperature treatments were starved for 24 h. Following this starvation period, individual weights (g) were measured for all fish. On days 21 and 22 fish from the remaining 2 replicates of each treatment were starved for 24 h and sampled as described above.

4.3.4. Statistical Analysis

Data are presented as mean ± standard error. Relationships between feed intake, growth and growth efficiency and temperature were modelled using quadratic polynomials (SigmaPlot, version 8.0). Optimal ranges were determined as the estimated maximum ± one standard error.

Table 4.1. Nominal and measured temperature, initial and final body weight and survival (mean ± standard error) for juvenile barramundi in experiments 1 and 2.

	Temperature (°C)		Mean Body Weight (g)		Survival (%)
	Nominal	Measured	Initial	Final	
Experiment 1					
	21	20.6 ± 0.51	2.44 ± 0.05	3.27 ± 0.11	100.00 ± 0.00
	24	24.0 ± 0.02	2.66 ± 0.07	4.54 ± 0.18	100.00 ± 0.00
	27	27.3 ± 0.09	2.68 ± 0.08	11.99 ± 0.50	96.67 ± 1.67
	30	30.1 ± 0.02	3.44 ± 0.09	15.32 ± 0.56	98.33 ± 1.67
	33	32.5 ± 0.04	3.60 ± 0.08	16.18 ± 0.71	95.00 ± 5.00
Experiment 2					
	27	26.98 ± 0.04	4.23 ± 0.13	13.11 ± 0.45	98.33 ± 1.67
	33	33.16 ± 0.13	5.06 ± 0.19	22.47 ± 1.71	96.67 ± 1.67
	36	35.36 ± 0.32	4.49 ± 0.12	20.09 ± 1.76	98.33 ± 1.67
	39	38.86 ± 0.05	5.69 ± 0.17	10.33 ± 0.14	95.00 ± 2.89

Table 4.2. Body composition (mean ± standard error) of juvenile barramundi, *Lates calcarifer* in experiments 1 and 2. Means with similar or no superscrips were not significantly different ($p<0.05$, $n=3$) between temperatures. No comparison is made between the two experiments.

	Temperature (°C) Nominal	Dry Matter ($g\ kg^{-1}$)	Crude Protein ($g\ kg^{-1}$ WW)	Crude Lipid ($g\ kg^{-1}$ WW)	Ash ($g\ kg^{-1}$ WW)	Energy ($MJ\ kg^{-1}$ WW)
Experiment 1						
	21	$275.7^{b} \pm 2.47$	$158.0^{a,b} \pm 2.53$	61.6 ± 2.05	$39.0^{a,b} \pm 0.72$	5.77 ± 0.15
	24	$265.8^{a} \pm 2.22$	$146.0^{a} \pm 3.74$	54.6 ± 3.57	$40.3^{b} \pm 0.99$	5.53 ± 0.06
	27	$271.6^{a,b} \pm 0.68$	$159.6^{a,b} \pm 1.41$	58.3 ± 2.77	$37.6^{a} \pm 0.27$	5.62 ± 0.06
	30	$270.1^{a,b} \pm 1.59$	$160.2^{a,b} \pm 4.04$	56.2 ± 1.43	$39.2^{a,b} \pm 0.42$	5.79 ± 0.07
	33	$272.9^{b} \pm 1.23$	$162.5^{b} \pm 1.31$	56.1 ± 1.47	$37.6^{a} \pm 0.32$	5.72 ± 0.03
Experiment 2						
	27	$262.5^{b} \pm 0.17$	$154.9^{b} \pm 1.01$	$63.5^{a} \pm 1.27$	$37.8^{b} \pm 0.37$	$57.6^{a,b} \pm 0.67$
	33	$275.8^{a} \pm 0.14$	$164.2^{a} \pm 1.25$	$63.1^{a,b} \pm 2.42$	$39.6^{b} \pm 0.78$	$59.6^{a} \pm 0.95$
	36	$275.6^{a} \pm 0.08$	$163.8^{a} \pm 1.54$	$64.4^{a} \pm 2.96$	$42.1^{a,b} \pm 1.31$	$60.9^{a} \pm 0.83$
	39	$263.0^{b} \pm 0.23$	$147.4^{c} \pm 1.29$	$54.0^{b} \pm 2.39$	$44.2^{a} \pm 1.53$	$54.8^{b} \pm 0.93$

4.4. RESULTS AND DISCUSSION

This is the first time that feed intake, growth and growth efficiency data have been examined for juvenile barramundi over their thermal tolerance range. Barramundi provided an interesting species for these experiments, having a wide thermal tolerance for growth efficiency (Katersky and Carter, 2005), a large geographic distribution as well as being an important aquaculture species. Fish in experiment 1 had a four fold increase in body weight at temperature above 27°C, while in experiment 2 this magnitude of increase in body weight was only seen at temperatures 33 and 36°C (Table 4.1). In both experiments, mean treatment survival was high, above 95% (Table 4.1).

There were no significant relationships between temperature and whole-body chemical composition (crude protein ($r^2 = 0.266$; $F_{2,6} = 1.09$; $p = 0.395$), crude lipid ($r^2 = 0.024$; $F_{2,6} = 0.074$; $p = 0.929$) and energy ($r^2 = 0.128$; $F_{2,6} = 0.44$; $p = 0.663$)). The effect of temperature on whole body composition is unclear in the literature. It has been shown that body composition parameters are affected mainly by fish size, diet and life-history stage (Shearer, 1994; Shearer, *et al.*, 1994; Koskela, *et al.*, 1997; Van Ham, *et al.*, 2003) and temperature directly affects the feed intake and growth of fish, it apparently has little direct effect on the composition of the weight gained (Koskela, *et al.*, 1997). Studies have shown that whole-body crude protein was significantly affected by temperature, whilst others have shown that whole body crude lipid decreases with temperature (reviewed by Shearer, 1994) and at low temperatures, an increase in crude lipid (even with reduced rations) was attributed to low metabolic rate. As the temperature increased, the fish were unable to consume enough energy to maintain the higher proportion of the diet which is going to maintenance requirements and therefore unable to accumulate lipid into the whole-body (Shearer, 1994).

The relationships generated from these models provided an optimal temperature and an optimal range for each parameter. The relationship between feed intake (%·d^{-1}) and temperature (T°C) was described by:

$$FI\ (\%\cdot d^{-1}) = -60.29 + 4.19T - 0.06T^2\ (r^2 = 0.801;\ F_{2,6} = 12.06;\ p = 0.008) \qquad (4)$$

This model predicts that maximum feed intake occurred at 31.4 °C (Fig. 4.1a) and the range for maximum intake was between 30.9 and 31.9°C. The relationship between growth (SGR) and temperature was described by:

$$SGR\ (\%\cdot d^{-1}) = -51.69 + 3.73T - 0.06T^2\ (r^2 = 0.862;\ F_{2,6} = 18.75;\ p = 0.003) \qquad (5)$$

According to this model peak growth occurred at 31.4 °C (Fig. 4.1b) and the range for maximum growth was between 30.5 and 32.4 °C. The optimal temperature for feed intake has generally been shown to be a few degrees higher than that for maximum growth (reviewed by Jobling, 1997; McCarthy, *et al.*, 1998; Jonsson, *et al.*, 2001) however, these models predict maximum feed intake and maximum growth occur at the same temperature. Models for Atlantic salmon show similar results with optimal feed intake and growth being within 1°C of each other (Forseth, *et al.*, 2001).

The narrow optimal range for both feed intake and growth can be attributed to metabolic demand. As temperature increases within the thermal tolerance range, metabolism will increase exponentially in small juvenile fish (Jobling, 1997). As temperature increases, the dissolved oxygen (DO) in the water decreases. Jobling (1997) suggested that at high temperature, with low DO, it is possible that the appetite is reduced due to the respiratory and circulatory systems inability to deliver oxygen to respiring tissues under high oxygen demand. As the size of the fish increase the routine oxygen consumption will generally increase, however on a weight-specific basis small fish consume more oxygen than their larger conspecifics (reviewed by Jobling, 1994). This relationship has been identified in barramundi and

when the effect of temperature was introduced it became apparent that larger fish may be more susceptible to problems associated with low DO than smaller fish due to high routine oxygen consumption (Glenncross and Felsing, 2006). Furthermore, the post-prandial increase in metabolism can be nearly double routine metabolism (Jobling, 1994; Katersky, *et al.*, 2006) and can remain elevated for long periods of time, depending on temperature and meal size (Jobling, 1994). The added effect of post-prandial metabolic increase causes a further strain to an already stressed system at high temperatures and may be the determining factor as to whether the fish is able to continue to feed. The cumulative effect of increased metabolic demand and decreased oxygen availability consequently limit the growth potential of fish as temperature increases past the optimum (Jobling, 1997; Koskela, *et al.*, 1997).

The relationship between protein growth efficiency (PPV) and temperature was described by:

$$PPV\ (\%) = -265.05 + 20.06T - 0.32T^2\ (r^2 = 0.824;\ F_{2,6} = 14.03;\ p = 0.005) \qquad (6)$$

From this model peak efficiency occurred at 31.2 °C (Fig. 4.1c), and maximum protein growth efficiency is estimated to occur at temperatures ranging from 25.3 to 37.0 °C. The relationship between energy growth efficiency (PEV) and temperature was described by:

$$PEV\ (\%) = -208.46 + 16.08T - 0.26T^2\ (r^2 = 0.803;\ F_{2,6} = 12.25;\ p = 0.008) \qquad (7)$$

This model shows that the peak productive energy value occurred at 31.3 °C (Fig. 4.1d) and that maximum rates of efficiency occur between temperatures 26.2 and 36.4 °C. Under optimal conditions, the PPV and PEV ranged from 36.3-47.4 % and 36.4-43.1%, respectively. These values are similar to PPV and PEV values found for larger barramundi (59 and 176 g), 38 and 43 %, 40 and 39 % at 29°C, respectively (Williams, *et al.*, 2006). Maximum growth efficiency is dependant upon a balanced

diet providing the appropriate protein to energy ratios and essential amino acids (reviewed by Wilson, 2002). In the present experiments, diet formulation was based upon the protein and energy requirements known to promote optimal growth and it is clear that the barramundi were able to utilize the protein and energy available in the diet and therefore maintain maximum efficiency over a wide range of temperatures. Increasing protein to energy ratios above the dietary requirement, results in increased ammonia excretion and protein synthesis (reviewed by Carter and Houlihan, 2001). Unfortunately increased synthesis does not translate into increased protein retention because synthesised proteins are degraded and catabolized, this in turn increasing energy expenditures and decreasing the amount of energy available for growth (reviewed by Bowen, 1987; Carter and Houlihan, 2001). As protein to energy ratios increase for juvenile barramundi, the relationship with feed conversion and growth rate level off once the optimal ratio (26.7 g CP•MJ GE^{-1}) is reached (reviewed by Boonyaratpalin and Williams, 2002; Glencross, 2006). Furthermore, imbalances in dietary essential amino acids can cause greater oxidation of amino acids and decrease growth efficiency in fish (Williams, *et al.*, 2001; Conceicao, *et al.*, 2003). This is apparent in a series of experiments where barramundi were fed varying levels of crystalline- and protein-bound amino acids (Williams, *et al.*, 2001). In relation to dietary lysine, growth efficiency and lysine retention decreased as lysine concentration increased past the optimal level for both forms of amino acids (Williams, *et al.*, 2001).

The magnitude of the range for maximum growth efficiency (PPV and PEV) is 10.9°C, this is ~10 times greater than the magnitude of the range for SGR. These patterns are also evident in models which have been developed for several fish, Atlantic salmon (Forseth, *et al.*, 2001), common wolfish, *Anarhichas lupus* (McCarthy, *et al.*, 1998) and European sea bass, *Dicentrarchus labrax* (Person-Le Ruyet, *et al.*, 2004) and show that growth efficiency plateaus over a wider range of temperatures than for feed intake or growth. In the present study, the magnitude of the optimal range was much greater than previously recognized for juvenile

barramundi when the entire thermal tolerance range was examined. This is a possibly a biological adaptation fish develop in order to adapt to different thermal environments throughout their life history (Imsland, *et al.*, 1996; Jobling, 1997; Duston, *et al.*, 2004). This is especially important for small juvenile barramundi migrating from coastal estuaries to shallow inland rivers at high temperatures in Australia and Southeast Asia. Maintaining high growth efficiency under the wide range of temperatures would be essential for survival a time when rapid growth is occurring. This also confirms how robust barramundi are for aquaculture development outside their natural geographic distribution, with efficient culture possible under a wide range of temperatures.

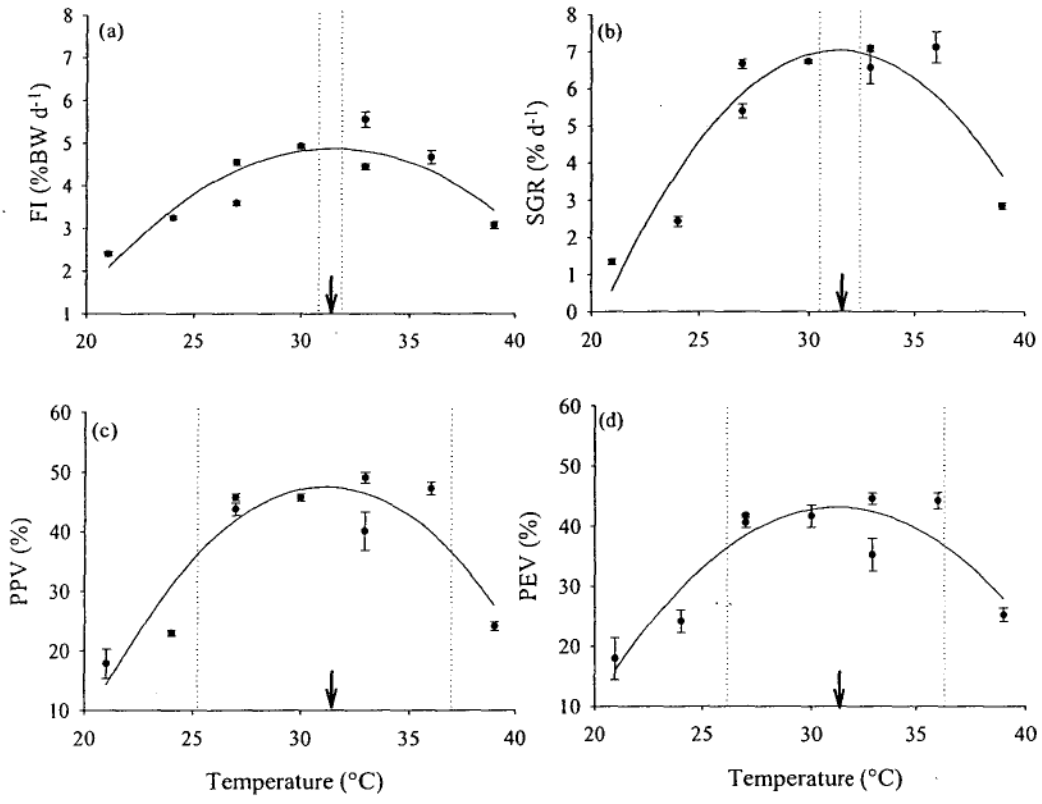


Figure 4.1. The relationship between temperature and (a) feed intake (FI, %BW•d^{-1}), (b) specific growth rate (SGR, %•d^{-1}), (c) productive protein value (PPV, %) and (d) productive energy value (PEV, %) for juvenile barramundi, *Lates calcarifer*. Each relationship is described with a quadratic polynomial. The arrow indicates the temperature where the maximum estimate occurs (optimal temperature) and the vertical lines indicate the standard error of the maximum estimate (optimal range).

4.5. REFERENCES

Boonyaratpalin, M., Williams, K., 2002. Asian Sea Bass, *Lates calcarifer*. In: Webster, C.D., C., L. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, pp. 40-50.

Bowen, S.H., 1987. Dietary protein requirements of fishes- A reassessment. Canadian Journal of Fisheries and Aquatic Sciences 44, 1995-2001.

Brett, J.R., Groves, T.D.D., 1979. Physiological Energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Vol. VIII: Bioenergetics and Growth. Acedemic Press, New York, NY, pp. 279-351.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Wright, P., Anderson, P. (Eds.), Fish Physiology, Vol. 20: Nitrogen Excretion. Academic Press, New York, NY, pp. 31-75.

Conceicao, L.E.C., Grasdalen, H., Ronnestad, I., 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. Aquaculture 227, 221-232.

Duston, J., Astatkie, T., MacIsaac, P.F., 2004. Effect of body size on growth and food conversion of juvenile striped bass reared at 16-28 °C in freshwater and seawater. Aquaculture 234, 589-600.

Elliott, J.M., 1982. The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comparative Biochemistry and Physiology Part B 73B, 81-91.

Forseth, T., Hurley, M.A., Jensen, A.J., Elliott, J.M., 2001. Functional models for growth and food consumption of Atlantic salmon parr, *Salmo salar*, from a Norwegian river. Freshwat. Biol. 46, 173-186.

Glencross, B., 2006. The nutritional management of barramundi, *Lates calcarifer* - a review. Aquaculture Nutrition 12, 291-309.

Glenncross, B., Felsing, M., 2006. Influence of fish size and water temperature on the metabolic demand for oxygen by barramundi, Lates calcarifer (Bloch), in freshwater. Aquaculture Research 37, 1055-1062.

Houlihan, D.F., 1991. Protein turnover in ectotherms and its relationship to energetics. In: Gilled, R. (Ed.), Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin Heidelberg, pp. 1-43.

Imsland, A.K., Sunde, L.M., Folkvord, A., Stefansson, S.O., 1996. The interaction of temperature and fish size on growth of juvenile turbot. Journal of Fish Biology 49, 926-940.

Jobling, M., 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. Journal of Fish Biology 18, 87-96.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Jonassen, T.M., Imsland, A.K., Stefansson, S.O., 1999. The interaction of temperature and fish size on growth of juvenile halibut. Journal of Fish Biology 54, 556-572.

Jonsson, B., Forseth, T., Jensen, A.J., Naesje, T.F., 2001. Thermal performance of juvenile Atlantic Salmon, *Salmo salar* L. Functional Ecology 15, 701-711.

Katersky, R.S., Carter, C.G., 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Katersky, R.S., Peck, M.A., Bengtson, D.A., 2006. Oxygen consumption of newly settled summer flounder, *Paralichthys dentatus* (Linnaeus, 1766). Aquaculture 257, 249-256.

Koskela, J., Pirhonen, J., Jobling, M., 1997. Feed intake, growth rate and baltic composition of juvenile Baltic salmon exposed to different constant temperatures. Aquaculture International 5, 351-360.

McCarthy, I., Moksness, E., Pavlov, D.A., 1998. The effects of temperature on growth rate and growth efficiency of juvenile common wolffish. Aquaculture International 6, 207-218.

Person-Le Ruyet, J., Mahe, K., N., L.B., Le Delliou, H., 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquaculture 237, 269-280.

Roberts, R.J., Hardy, R.W., Sugiura, S.H., 2001. Screamer disease in Altantic salmon, *Salmo salar* L., in Chile. Journal of Fish Diseases 24, 543-549.

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119, 63-88.

Shearer, K.D., Asgard, T., Andorsdottir, G., Aas, G.H., 1994. Whole body elemental and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle. Journal of Fish Biology 44, 785-797.

Tucker, J., J.W., , Russel, D.J., Rimmer, M.A., 2002. Barramundi Culture: A success story for aquaculture in Australia, World Aquaculture, pp. 53-59.

Van Ham, E.H., Berntssen, M.H.G., Imsland, A.K., Parpoura, A.C., Bonga, S.W., Stefansson, S.O., 2003. The influence of temperature and ration on growth, feed conversion, body composition and nutrient retention of juvenile turbot (*Scophthalmus maximus*). Aquaculture 217, 257-558.

Wallace, J., 1993. Evironmental considerations. In: Heen, K., Monahan, R.L., Utter, F. (Eds.), Salmon Aquaculture. Halsted Press, New York, N.Y., pp. 127-143.

Williams, K., Barlow, C., Rogers, L., 2001. Efficacy of crystalline and protein-bound amino acids for amino acid enrichment of diets for barramundi/Asian seabass (*Lates calcarifer* Bloch). Aquaculture Research 32, 415-429.

Williams, K., Barlow, C.G., Rodgers, L., Agcorpra, C., 2006. Dietary composition manipulation to enhance the performance of juvenile barramundi (*Lates calcarifer*) reared in cool water. Aquaculture Research 37, 914-927.

Wilson, R.P., 2002. Amino acids and proteins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Pres, San Diego, pp. 144-179.

CHAPTER 5

THE EFFECT OF TEMPERAUTRE ON POST-PRANDIAL PROTEIN SYNTHESIS IN JUVENILE BARRAMUNDI, *LATES CALCARIFER*

5.1. ABSTRACT

The experiment aimed to measure post-prandial protein synthesis at three different temperatures. Juvenile barramundi (10.81 ± 3.46 g) were held at 21, 27 and 33°C and fed to satiation daily (504.5 g kg^{-1} crude protein, 156 g kg^{-1} lipid, 128.5 g kg^{-1} ash, 20.1 GE MJ kg^{-1}). Samples were taken over a 24 h period at 0 (24 h after the previous meal) and then 4, 8, 12 and 24 h after feeding to measure protein synthesis in the white muscle, liver and remaining carcass. Protein synthesis at 27 and 33°C peaked 4 h after feeding in all tissues and retuned to pre-feeding rates by 12 h. At 21°C protein synthesis remained constant over 24 h in all tissues and was attributed to the low feed intake and low growth. While the concentration of RNA remained stable over the 24 h cycle and across temperatures, the ribosomal activity increased after feeding. This meant k_{RNA}, not the absolute amount of RNA, was the driving force underlying the post-prandial increase in protein synthesis. However, relative differences in protein synthesis between tissues were attributed to differences in RNA concentration. There was a significant positive relationship between white muscle and whole body protein synthesis, white muscle could be used as a predictor of whole body protein synthesis across temperature and the time after feeding. This was the first study to show an interaction between temperature and the time after feeding for an ectotherm and that post-prandial peak only occurred under optimum temperature conditions.

Keywords: *Lates calcarifer*; Post-prandial metabolism; Protein synthesis; RNA; Specific dynamic action; temperature

5.2. INTRODUCTION

Protein synthesis occurs in all living organisms and is the underlying process driving growth. Many factors, both biotic and abiotic, will affect this process. Temperature has been identified by numerous researchers as the key abiotic factor that controls the growth rate of ectotherms (reviewed by Brown, 1957; Brett and Groves, 1979; Elliott, 1994; Jobling, 1994; 1997; McCarthy and Houlihan, 1997). It is generally accepted that protein synthesis will increase with increasing temperature when an unlimited supply of food is available and the temperature increase is within the thermal tolerance range (reviewed by Houlihan, 1991; McCarthy and Houlihan, 1997). The relationships between temperature, feed intake, growth and protein synthesis are interrelated. Temperature drives metabolic demand and therefore feed intake which in turn drives protein synthesis and growth. The approach taken here was to measure the response to *ad libitum* feeding. Protein synthesis occurs in all tissues at varying rates with the liver and gills having the highest rate of synthesis and the white muscle the lowest (reviewed by Carter and Houlihan, 2001). Synthesis rate of specific tissues depends on metabolic function, consequently the liver has high rates due to synthesis of enzymes and of proteins for export to other tissues. The contribution that the liver and white muscle make to whole body protein synthesis is significant, albeit for different reasons and the combination of tissue protein mass and rate of synthesis means that the white muscle and liver account for the majority of whole body protein synthesis.

The cost of whole body protein synthesis has been shown to account for up to 42% of the energy expenditures for feeding fish (reviewed by Carter and Houlihan, 2001). After feeding there is a post-prandial increase in protein synthesis (McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992). This is part of specific dynamic action (SDA) that encompasses all physiological events associated with feeding including protein synthesis (reviewed by Carter, *et al.*, 2001; McCue, 2006). SDA is generally determined by measuring the time course of oxygen consumption after a meal and

post-prandial increases in oxygen consumption correspond with increases in protein synthesis (reviewed by Houlihan, 1991). Oxygen consumption increases with increased temperature and is reflected in a linear increase in tissue protein synthesis at temperatures within the thermal tolerance range (reviewed by McCarthy and Houlihan, 1997). The metabolic rate in the liver is high and the rapid response to feeding in protein synthesis are thought to determine when peak SDA occurs (reviewed by Jobling, 1983; McCue, 2006). Whilst white muscle protein synthesis may be the lowest of all tissues, it has the largest tissue mass and reflective of the whole body protein synthesis rates (reviewed by Carter and Houlihan, 2001) and therefore makes a significant contribution to SDA (Lyndon, *et al.*, 1992). Increased protein synthesis in the liver and white muscle with temperature have similar slopes indicating that there is a single temperature response regardless of the relative rates of protein synthesis (reviewed by McCarthy and Houlihan, 1997). The post-prandial effect of whole body protein synthesis is a composite of different tissues which respond to feeding at different times and this is reflected in the SDA (reviewed by Houlihan, 1991). By measuring the post-prandial protein synthesis in the white muscle, liver and whole body, it is possible to determine contribution of the liver and white muscle to whole body protein synthesis at various times after feed intake at a range of temperatures within the thermal tolerance range.

The process of protein synthesis can be defined as mRNA translation (Taylor and Brameld, 1999). The relationships between the RNA, RNA:Protein and ribosomal activity with protein synthesis has received attention as predictors of growth (reviewed by Houlihan, *et al.*, 1993) as well as to whether the concentration of RNA or the ribosomal activity which regulates protein synthesis. The current view on the relationship of RNA to protein synthesis is that, differences in RNA concentrations exist between tissues and these differences are directly related to the relative differences seen in protein synthesis rates between tissues (McMillan and Houlihan, 1988; 1989; Lyndon, *et al.*, 1992), fish at low acclimation temperatures will have higher concentrations of RNA to compensate for lower activity than fish at higher

acclimation temperatures (Foster, *et al.*, 1992) and that the post-prandial effect is driven by increases in ribosomal activity stimulated by feed intake (McMillan and Houlihan, 1989). Currently, there is no information concerning the interaction of temperature and feed intake on the mechanisms of protein synthesis over a 24 h period and is of interest in determining how RNA regulates protein synthesis. The impetus behind this research was to understand how the pattern of protein synthesis changed over the initial 24 h after feeding in the liver, white muscle and the whole body at three temperatures which extend across the thermal tolerance for juvenile barramundi. The mechanisms of protein synthesis are also considered in determining the driving force of the daily cycle of protein synthesis under different thermal conditions. With its wide thermal tolerance range, barramundi (*Lates calcarifer*) are a useful tropical model species to examine the effect of temperature on daily rates of protein synthesis. This species provides an interesting opportunity to understanding how environmental temperature affects the protein metabolism of an ectothermic animal.

5.3. MATERIALS AND METHODS

5.3.1. Diet

A standard diet was formulated according to known dietary requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet was formulated to contain 50% crude protein and 19.7 MJ kg^{-1} gross energy (Table 5.1). Fish meal and fish oil were supplied by Skretting (Cambridge, TAS, Australia). Vitamins and minerals were supplied individually by Sigma-Aldrich Pty. Ltd. (Sydney, NSW, Australia) and two pre-mixes were made on-site, Vitamin C was supplied as Stay-C from Roche Pharmaceuticals (Roche Vitamins Australia Ltd., Sydney, NSW, Australia).

5.3.2. Acclimation Period

Juvenile barramundi (1-2 g) were obtained from WBA Hatcheries (South Australia, Australia). Fish were maintained at the University of Tasmania under constant environmental conditions (salinity: 10 ‰; photoperiod: 24 h light; temperature: 27°C). Fish were stocked into 5 150-L aquaria and maintained at 27°C until they reached approximately 10 g. The fish were then randomly separated into three identical recirculation systems each consisting of 3 19-l carboys with an individual trickle biofilter on each system (Katersky and Carter, 2005). Temperatures were adjusted 1°C d^{-1} towards there experimental temperatures of 21 and 33°C, with the exception of 27°C system which was maintained at a constant temperature. After 6 days all fish were at their experimental temperature. Each system was then held at a constant temperature (21, 27 or 33°C) with submersible heaters each controlled by an individual thermostat. All fish were fed the standard diet to satiation once daily at 1200 for 7 days. Growth was not measured in this experiment.

5.3.3. Sampling Protocol

Samples were taken at time 0 (24 h after the previous meal) and then 4, 8, 12 and 24 h after feeding in order to measure rates of protein synthesis in the white muscle (WM), liver and remaining carcass. At each time, 6 fish were sampled (2 fish from each replicate tank) per treatment.

5.3.4. Protein Synthesis

Rates of protein synthesis were measured following a single injection of ^{3}H-phenylalanine using the flooding-dose method (Garlick, *et al.*, 1980; Houlihan, *et al.*, 1986). Barramundi were anesthetized with benzocaine (100 mg·L^{-1}), weighed and injected via the caudal vein with ^{3}H-phenlyalanine at a concentration of 1ml·100g body $weight^{-1}$. The injection solution contained 150 μmol L-phenylalanine and L-[2,6-^{3}H]phenylalanine (Amersham Pharmacia Biotech, NSW, Australia) in 0.2 μm

filtered seawater at pH 7.4 (Garlick, *et al.*, 1980). The measured specific activity of the injection solution was 1123 ± 118 dpm $nmol^{-1}$ phenylalanine.

Following injection, fish were returned to separate aquaria containing aerated water (10‰). Incorporation times of ~60 min were established in previous experiments (Chapter 2), and a time course was not necessary. Following incorporation fish were removed from the tank, killed by an overdose of benzocaine (400 $mg \cdot L^{-1}$) and transection of the spinal cord. Individual samples of white muscle (WM), liver and the remaining carcass from each temperature treatment were frozen in liquid nitrogen for later analysis. The subsequent analysis of samples to measure protein-bound and free-pool phenylalanine-specific radioactivities was as described previously (Houlihan, *et al.*, 1988; Houlihan, *et al.*, 1995a). Briefly, protein concentrations were measured using a modification of the folin-phenol method (Lowry, *et al.*, 1951) and RNA concentrations ([RNA]) were measured using dual wavelength absorbance (Ashford and Pain, 1985). RNA was also expressed as the capacity for protein synthesis (Cs: mg RNA · g $protein^{-1}$) and as RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$) (Sugden and Fuller, 1991).

Fractional rates of protein synthesis for WM, liver and carcass (k_s, $\% \cdot d^{-1}$) were calculated according to the following formula:

$$k_s = (S_b/S_a) \cdot (1440/t) \cdot 100 \qquad (1)$$

where S_b is the protein bound phenylalanine specific radioactivity; S_a is the free-pool phenylalanine specific radioactivity; t is the post injection incubation time in minutes, (Garlick, et al., 1980). Liver k_s was calculated using a mean free-pool for each temperature, no significant difference was found among liver S_a values over the course of the day within each temperature treatment (21°C: F=0.49; df=1,28; p=0.49; 27°C: F=0.12; df=1,27; p=0.73; 33°C: F=0.02; df=1,26; p=0.88).

For each fish a whole body (WB) rate of protein synthesis (k_s, $\%\cdot d^{-1}$) was calculated from the remaining carcass, liver and WM synthesis according to the following formula:

$$WB_{ks} = ((\sum k_{sWM}\cdot gP^{-1} + k_{sL}\cdot gP^{-1} + k_{sC}\cdot gP^{-1}) / g\ P) * 100 \quad (2)$$

Where, $k_{sWM}\cdot gP^{-1}$ is the WM $k_s \cdot$ g protein^{-1} in the WM; $k_{sL}\cdot gP^{-1}$ is the liver ks $\cdot$ g protein^{-1} in the liver; $k_{sC}\cdot gP^{-1}$ is the carcass ks $\cdot$ g protein^{-1} in the carcass; P is the total protein in the fish.

5.3.5. Statistical Analysis

Data are presented as mean ± standard error. The normality and homogeneity of data were explored by examining the residual plots. Results were analyzed using a two-way ANOVA (SPSS, version 11.5) to look at the effects of temperature and the time after feeding. Where there was a significant interaction a one-way ANOVA followed by a Tukey HSD were used to identify significantly different means. When interactions of these two factors were not significant the individual 1-way ANOVA results were examined and significant results were compared using Tukey's HSD.

Table 5.1. Ingredient and chemical composition of experimental diet.

Ingreadient Compostion (g · kg^{-1})	
Fish meal	730
Fish oil	70
Starch	119
CMC	10
Choline chloride	10
Phosphorus ($NaPO_4$)	10
Vitamin C (Stay-C)	20
Yetterbium-oxide	1
Vitamin premix[a]	15
Mineral premix[b]	15
Chemical Compostion (g · kg^{-1} DM)	
Dry matter (g·kg^{-1})	927.5
Crude protein	483.1
Crude lipid	163.9
Ash	120.6
Energy (MJ·kg^{-1})	19.74

[a]Vitamin premix (mg kg^{-1}): Vitamin A (7.50), Vitamin D (9.00), Rovimix E50 (150.00), Menadione sodium bisulphate (3.00), Riboflavin (6.00), Calcium D-pantothenate (32.68), Nicontinic Acid (15.00), Vitamin B-12 (0.015), d-biotin (0.23), Folic acid (1.50), Thiamin HCl (1.68), Pyridoxine HCl (5.49), myo-Inositol (450.00), α-cellulose (817.91).

[b]Mineral premix (mg kg^{-1}): $CuSO_4$ $5H_20$ (35.37), $FeSO_4$ $7H_2O$ (544.65), $MnSO_4$ H_2O (92.28), Na_2SeO_3 (0.99), $ZnSO_4$ $7H_2O$ (197.91), KI (2.16), $CoSO_4$ $7H_2O$ (14.31), α-cellulose (612.33).

5.4. RESULTS

5.4.1. Feed Intake

Feed intake (g) on the day of the 24 h time course experiment was significantly higher in the 27 and 33°C groups ($F=27.29$; $df=2,8$; $p<0.001$) than at 21°C. Feed intake (mean ± s.d.) at 21°C was 3.07 ± 0.47 g, at 27°C, 8.07 ± 0.50 g and at 33°C it was 10.10 ± 1.26 g (Table 5.2). Feed intake expressed as % body weight was significantly different between the three temperature groups ($F=44.59$; $df=2,8$; $p<0.001$, Table 5.2). When feed intake was expressed as the fractional rate of protein consumption (k_c, $\%\cdot d^{-1}$) significant differences were apparent between the temperature groups ($F=55.89$; $df=2,8$; $p<0.001$, Table 5.2).

5.4.2. Protein synthesis

5.4.2.1. White Muscle

There was a significant interaction ($F=3.54$; $df=8,68$; $p=0.002$) between temperature and the time after feeding for white muscle protein synthesis (Fig. 5.1a). Fish which were held at 27°C and 33°C showed significantly higher protein synthesis than the 21°C fish at 4, 8 and 12 hours after feeding. However there was no difference between the 27 and 33°C groups at any time. At 21°C protein synthesis did not change over the 24 h.

5.4.2.2. Liver

There was a significant interaction ($F=2.22$; $df=8,67$; $p=0.036$) between temperature and the time after feeding for protein synthesis in the liver (Fig. 5.1b). Fish held at 33°C had significantly higher protein synthesis at 4, 8 and 12 h after feeding than the 21°C fish and the 27°C fish at 12 h. Fish at 27°C had significantly higher protein

synthesis then the fish from the 21°C treatment at 4 h after feeding (Fig 5.1b). At 21 °C protein synthesis did not change over the 24 h.

5.4.2.3. Whole Body

There was a significant interaction ($F=2.53$; $df=8,80$; $p=0.018$) between temperature and the time after feeding for whole body protein synthesis (Fig 5.1c). Fish which were held at 33°C had significantly higher protein synthesis at 4, 8 and 12 hours after feeding than the 21°C fish, while protein synthesis at 27°C was not significantly different from 33°C and were only significantly different from the 21°C treatment at time 0 and 4 h after feeding. At 21°C protein synthesis did not change over 24 h. There was a significant positive linear relationship between the WM k_s and WB k_s ($r^2=0.703$; $F_{1,78}=187.63$; $p<0.001$, Fig 5.2) with WB k_s being 3 times the WM k_s, irrespective of temperature.

5.4.3. RNA correlates

5.4.3.1. White Muscle

There was no significant interaction ($F=1.99$; $df=4,68$; $p=0.11$) between temperature and the time after feeding for RNA concentration. However, there was a significant effect of temperature ($F=16.09$; $df=2,68$; $p<0.001$) on the RNA concentration (Table 5.2). Fish at 27 °C had significantly higher RNA concentrations than fish held at 21 and 33°C. There was a significant interaction ($F=2.54$; $df=8,69$; $p=0.02$) between temperature and the time after feeding for Cs, however, no differences were seen at any time point between the temperatures (Fig 5.3a). There was a significant interaction ($F=2.63$; $df=8,68$; $p=0.01$) between temperature and time for k_{RNA} (Fig. 5.4a). The k_{RNA} was significantly greater at 4 h after feeding for the 27 and 33 °C than at 21 °C. No significant change was seen at 21 °C group over the 24 h.

5.4.3.2. Liver

There was no significant interaction between temperature and the time after feeding (h) for either the RNA concentration ($F=0.86$; $df=8,66$; $p=0.56$, Table 5.2), Cs ($F=0.69$; $df=8,66$; $p=0.70$) or k_{RNA} ($F=1.86$; $df= 8,66$; $p=0.08$) in the liver. Time had a significant effect on the Cs ($F=2.56$; $df=4,66$; $p=0.046$, Fig 5.3b). Individually, temperature and the time after feeding showed a significant effect on k_{RNA}. As temperatures increased the k_{RNA} significant increased (Fig. 5.5a) and the k_{RNA} was significantly increased at 4, 8 and 12 h after feeding compared to times 0 and 24 (Fig. 5.5b).

5.4.3.3. Whole Body

There was no significant interaction between temperature and the time after feeding for the RNA concentration ($F=0.88$; $df=8,71$; $p=0.54$, Table 5.2) or for Cs ($F=0.35$; $df=8,71$; $p=0.94$). Individually, temperature and the time after feeding had a significant effect on Cs. Fish held at 27 °C had significantly higher Cs than the other temperature treatments ($F=21.97$; $df=2,71$; $p<0.001$, Fig. 5.6a). Cs was significantly higher at 8 h after feeding than at 12 and 24 h, however there was not a difference between time 0, 4 and 8 h after feeding ($F= 4.76$; $df=4,71$; $p=0.002$, Fig. 5.6b). There was a significant interaction ($F=2.72$; $df=8,71$; $p=0.01$) between temperature and the time after feeding for k_{RNA} (Fig. 5.4b). At 4 h after feeding, k_{RNA} was significantly higher for fish held at 27 and 33 °C then for the fish which were held at 21°C. There was no significant change in k_{RNA} at 21 °C over 24 h.

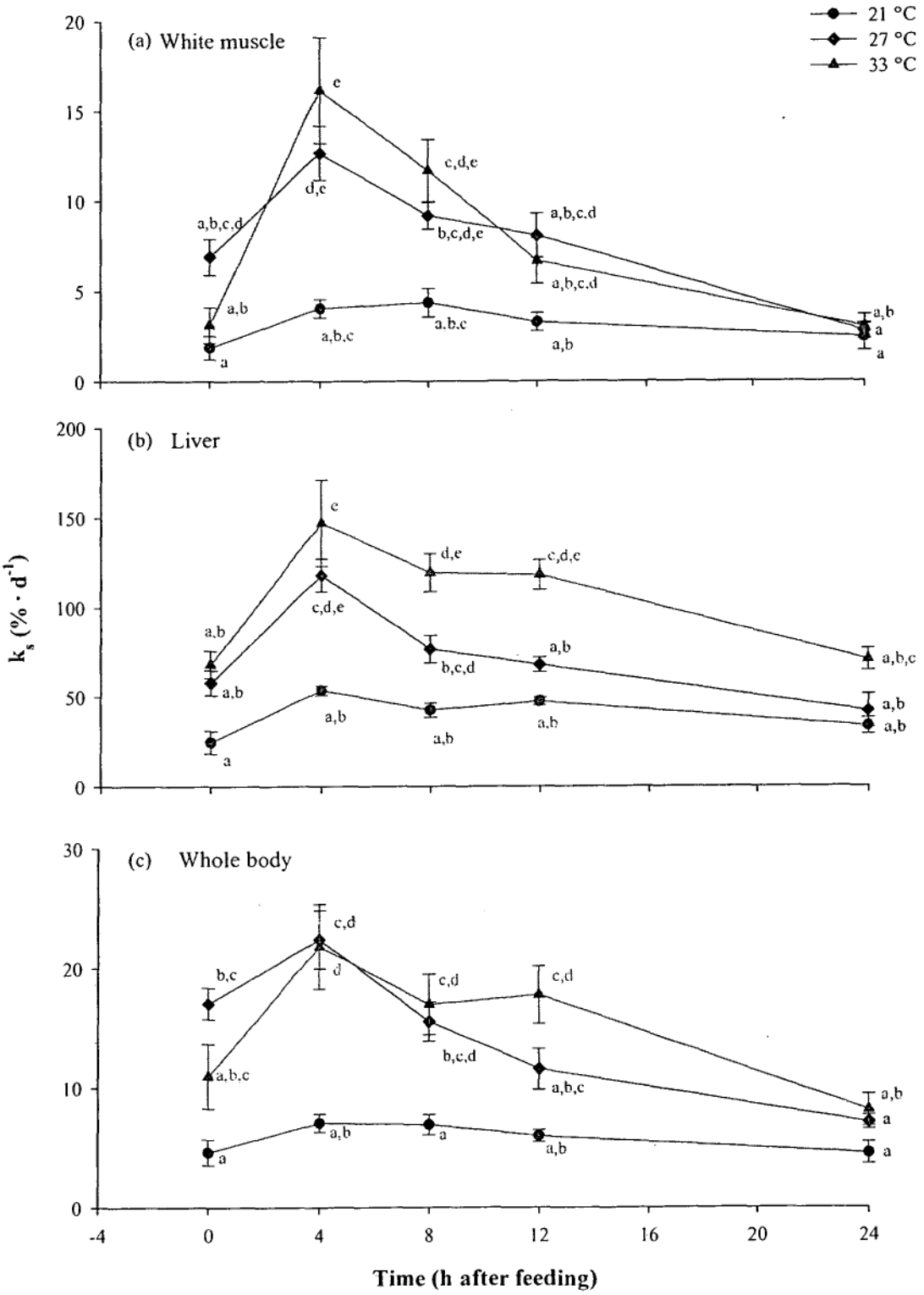
Table 5.2. The feed intake (mean ± s.d.) expressed as absolute consumption (g), % BW and k_c (% $\cdot d^{-1}$), mean fish weight, and concentration of RNA (μg RNA$\cdot mg^{-1}$) for the white muscle, liver and whole body at 21, 27 and 33°C for juvenile barramundi, *Lates calcarifer.*

Temperature (°C)	21	27	33
Fish Weight (g)	$11.31^{b} \pm 0.28$	$7.89^{c} \pm 0.31$	$13.15^{a} \pm 0.44$
Feed Intake			
Feed Intake (g)	$3.07^{b} \pm 0.47$	$8.07^{a} \pm 0.50$	$10.10^{a} \pm 1.26$
Feed Intake (% BW)	$0.96^{c} \pm 0.19$	$2.01^{b} \pm 0.28$	$3.87^{a} \pm 0.69$
k_c (% $\cdot d^{-1}$)	$4.35^{c} \pm 0.67$	$17.01^{a} \pm 1.28$	$11.89^{b} \pm 2.03$
RNA			
White muscle RNA (μg RNA$\cdot mg^{-1}$)	$1.41^{b} \pm 0.03$	$1.63^{a} \pm 0.04$	$1.39^{b} \pm 0.03$
Liver RNA (μg RNA$\cdot mg^{-1}$)	7.66 ± 0.32	8.01 ± 0.35	7.08 ± 0.18
Whole body RNA (μg RNA$\cdot mg^{-1}$)	$5.74^{b} \pm 0.15$	$6.83^{a} \pm 0.18$	$5.58^{b} \pm 0.18$

k_c (% $\cdot d^{-1}$), fractional rate of protein consumption = [g protein consumed $\cdot$ g fish protein$^{-1} \cdot d^{-1}$]*100

Means with similar or no superscripts ($p<0.05$, $n=3$) were not significantly different between temperatures.

Figure 5.1. The effect of feed intake on the fractional rate of protein synthesis (k_s, $\%\cdot d^{-1}$) in (a) white muscle, (b) liver and (c) whole body for juvenile barramundi, over 24 h after a final meal at 21, 27 and 33°C. Letters indicate significant 2-way interaction between the temperature and time after feeding for each tissue ($p<0.05$).


21 °C
27 °C
33 °C
(a) White muscle
(b) Liver
(c) Whole body
k_s (% · d^{-1})
Time (h after feeding)

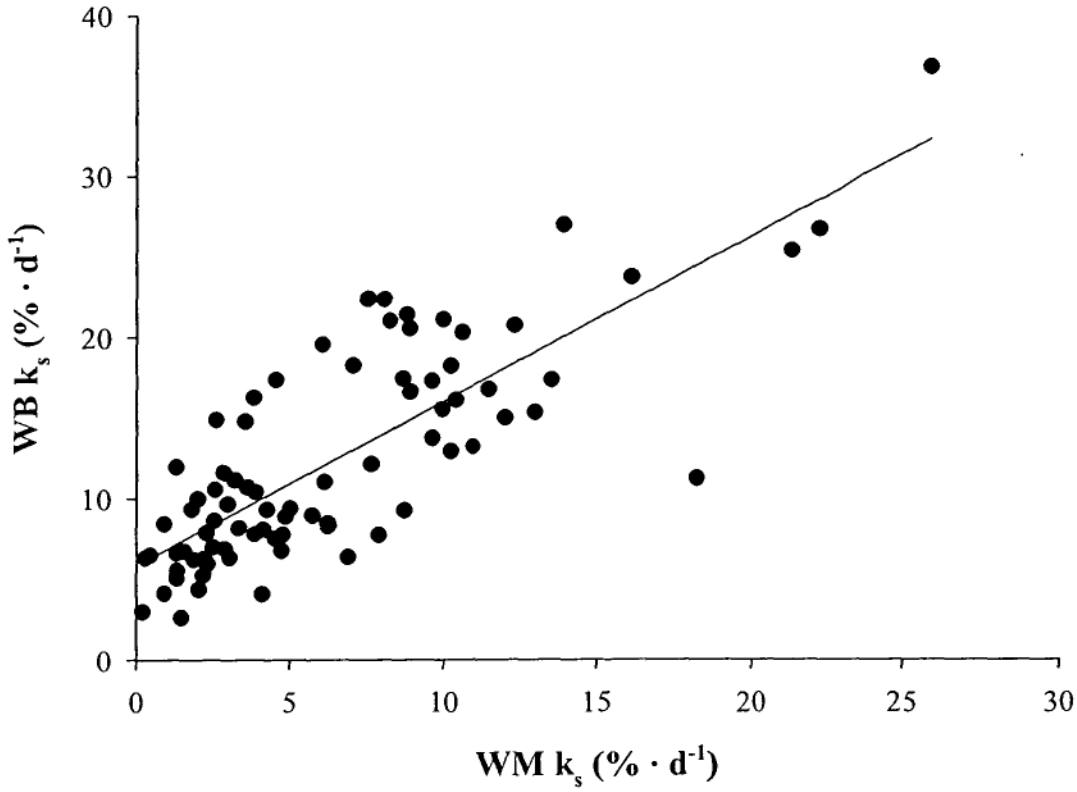
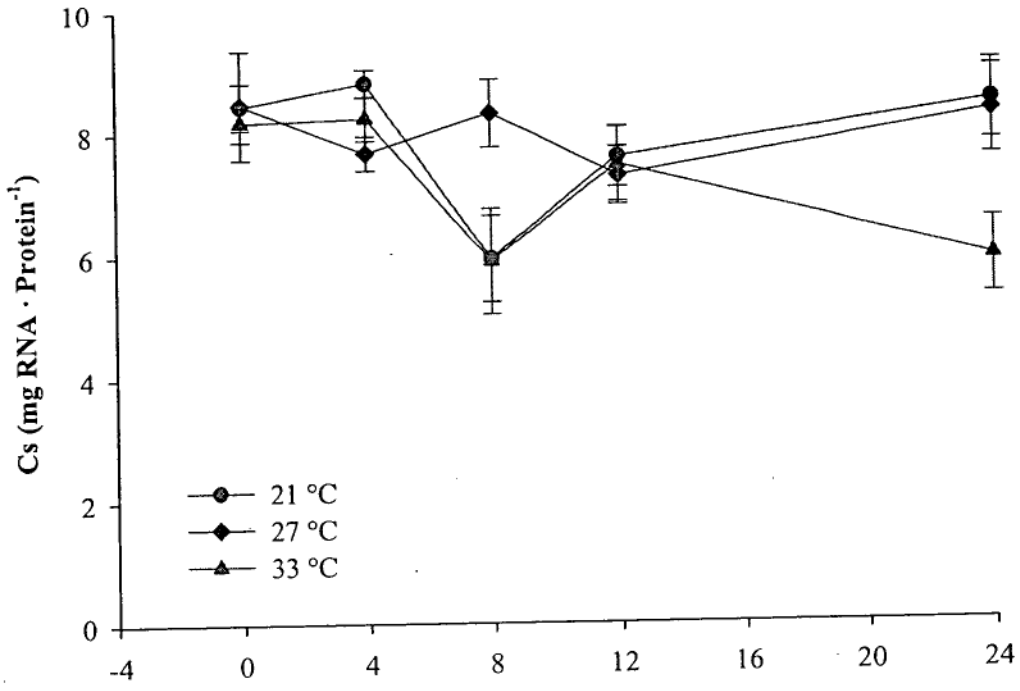


Figure 5.2. The relationship between white muscle protein synthesis (WM k_s) and whole body protein synthesis (WB k_s) in juvenile barramundi explained by the relationship, WB k_s = 1.018 ·WM k_s + 5.82 (r^2 = 0.703; $p<0.001$; n=79).

Figure 5.3. The effect of temperature and time on (a) white muscle RNA:Protein (Cs, mg RNA · g Protein^{-1}) in barramundi over 24 h. Different letters indicate a significant ($p<0.05$) two-way interaction between the temperature and time for white muscle Cs. (b) The effect of the time after feeding on the RNA:Protein (Cs, mg RNA · g Protein^{-1}) for the liver. Different letters indicate a significant difference between sample times ($p<0.05$).


Cs (mg RNA · Protein-1)
10
8
6
4
2
0
-4
0
4
8
12
16
20
24
21 °C
27 °C
33 °C

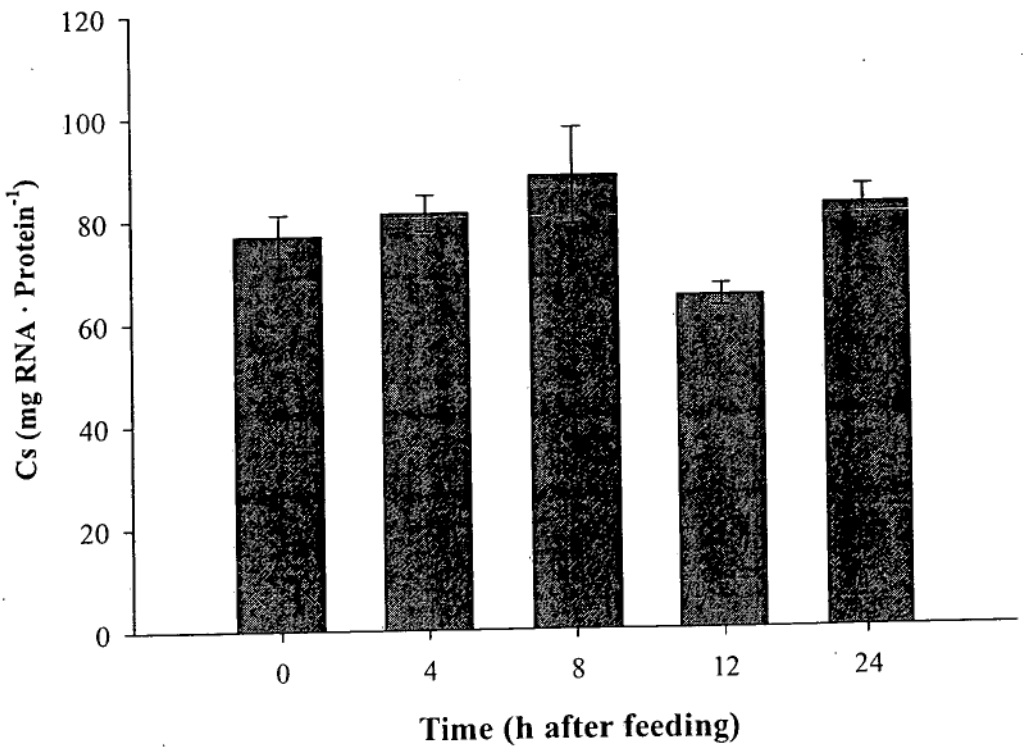

Cs (mg RNA · Protein-1)
120
100
80
60
40
20
0
0
4
8
12
24
Time (h after feeding)

Figure 5.4. The effect of feed intake on the RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA$\cdot d^{-1}$) in (a) white muscle and (b) whole body for juvenile barramundi over 24 h after a final meal at 21, 27 and 33°C. Letters indicate significant 2-way interaction between the temperature and time after feeding for each tissue ($p<0.05$).

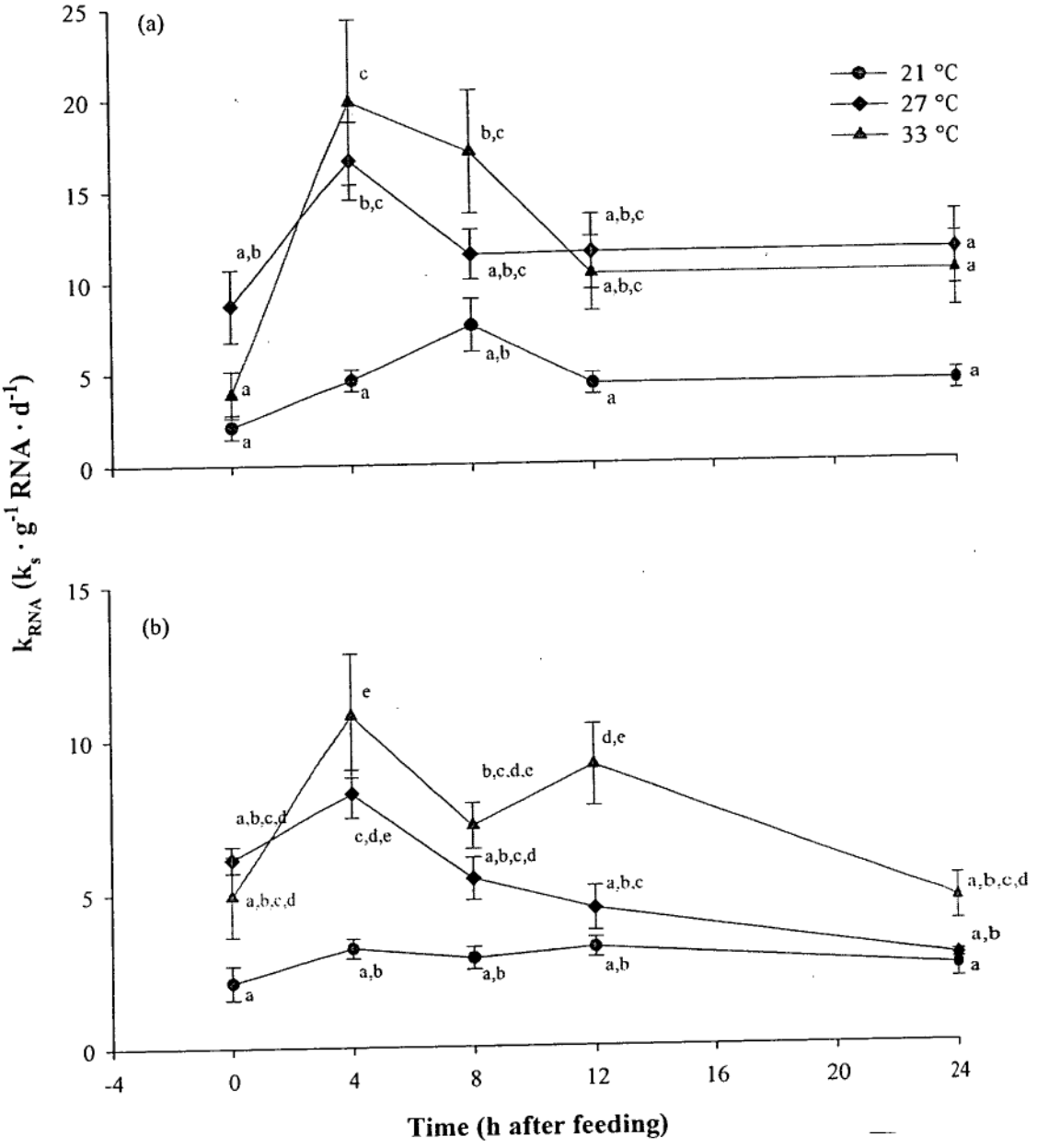
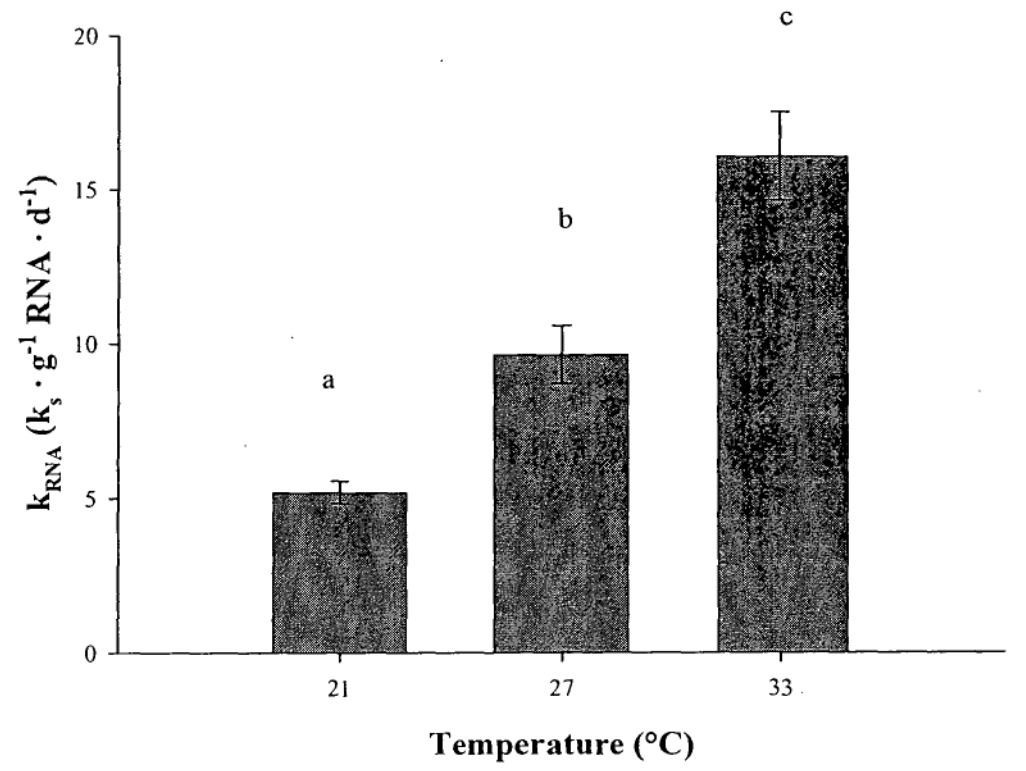

(a)
(b)
21 °C
27 °C
33 °C
k_{RNA} ($k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$)
Time (h after feeding)
0
5
10
15
20
25
-4
0
4
8
12
16
20
24
a,b
c
b,c
a,b,c
a,b,c
a
a
a
a
a
a
a,b
a
a
a,b,c,d
e
b,c,d,e
d,e
c,d,e
a,b,c,d
a,b,c
a,b,c,d
a,b
a
a,b
a,b
a,b
a

Figure 5.5. The effect of (a) temperature and (b) time after feeding on the RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA$\cdot d^{-1}$) in liver. Letters indicate a significant difference between groups ($p<0.05$).


20
15
10
5
0
k_{RNA} ($k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$)
a
b
c
21
27
33
Temperature (°C)

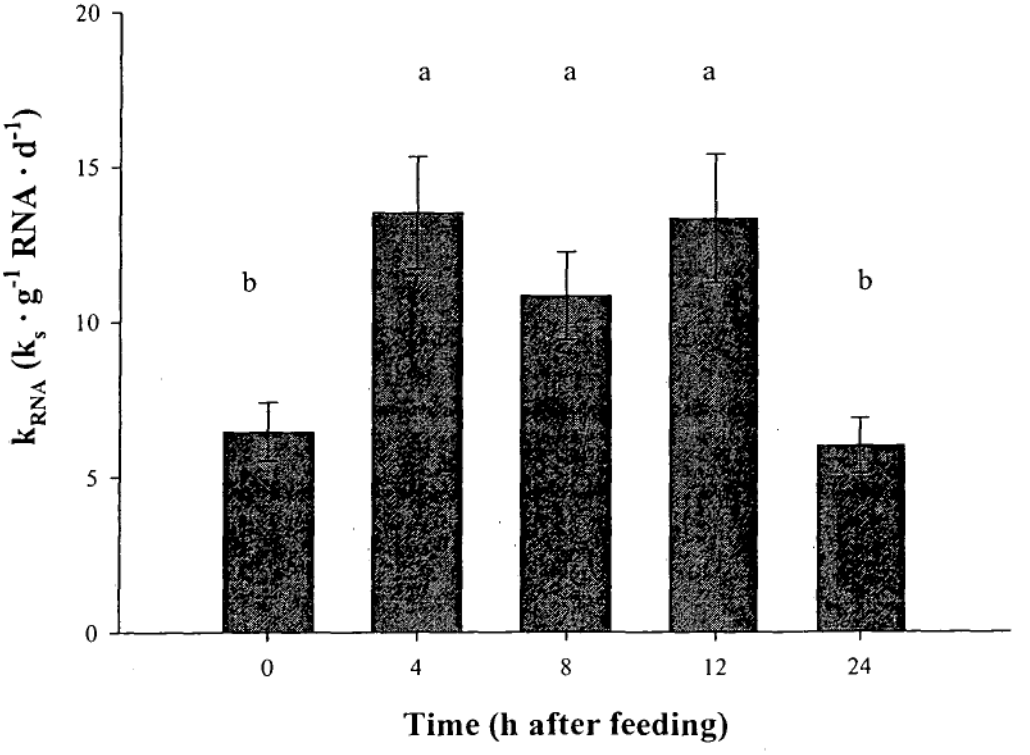
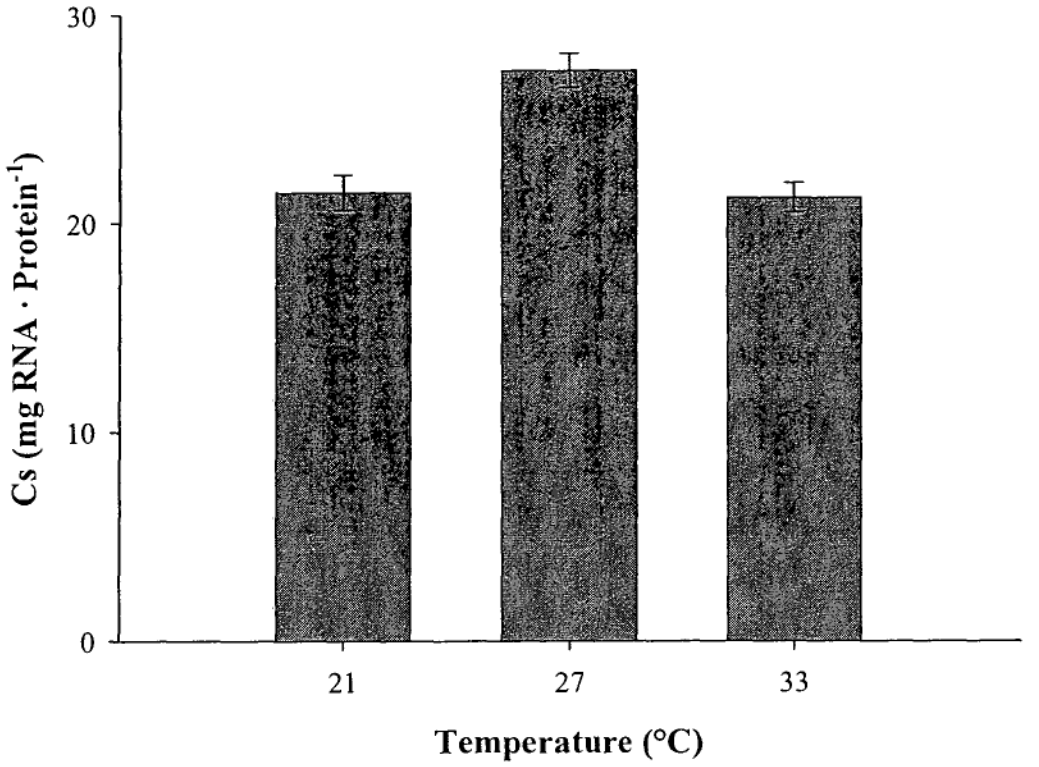
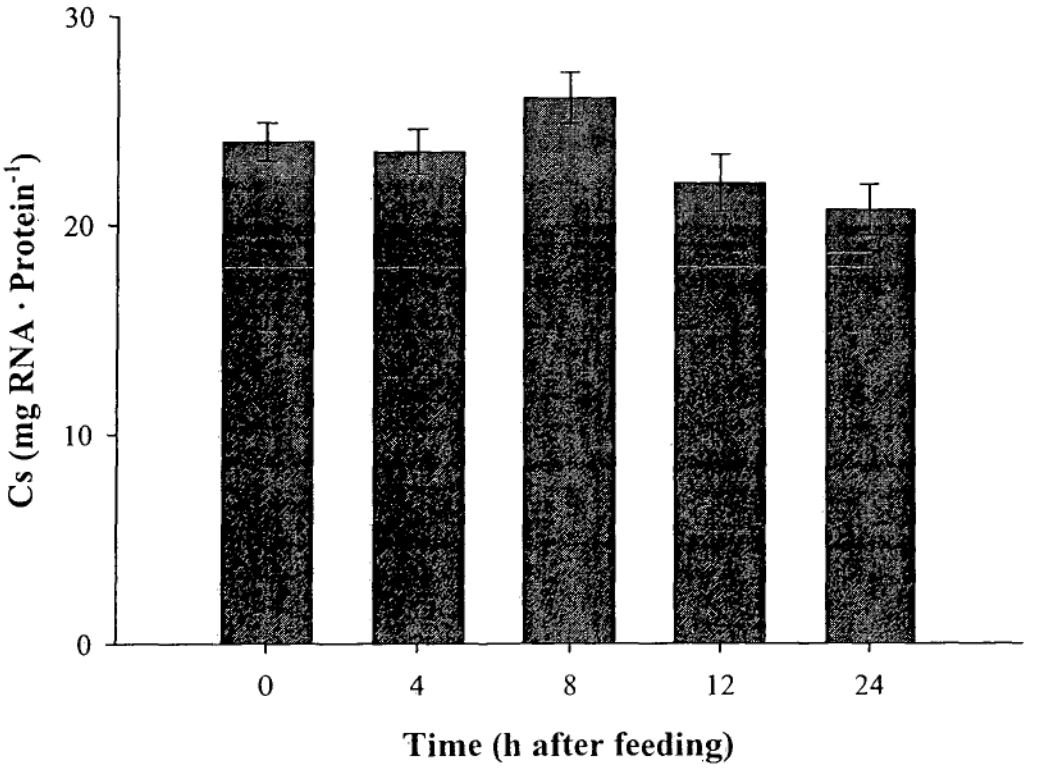

20
15
10
5
0
k_{RNA} ($k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$)
b
a
a
a
b
0
4
8
12
24
Time (h after feeding)

Figure 5.6. The effect of (a) temperature and (b) time after feeding on the RNA:Protein (Cs, mg RNA · Protein^{-1}) in the whole body for juvenile barramundi. Letters indicate a significant difference between groups ($p<0.05$).


30
20
10
0
Cs (mg RNA · Protein-1)
21
27
33
Temperature (°C)


30
20
10
0
Cs (mg RNA · Protein-1)
0
4
8
12
24
Time (h after feeding)

5.5. DISCUSSION

The present study was the first to examine the effects of temperature on the daily pattern of protein synthesis in whole fish in addition to different tissues. These were also the first measures of protein synthesis made over a 24 h period in barramundi, a model tropical ectotherm. The results showed that protein synthesis and RNA activity were affected by temperature and the time after feeding. There was a post-prandial effect at higher temperatures however, at the suboptimal temperature of 21°C protein synthesis was not stimulated by feeding. In the present study fish were fed to satiation, albeit from one feed per day, in order to examine effects at maximum feed intake. The relationship between feed intake and protein synthesis, irrespective of temperature, will be explored further in Chapter 7.

5.5.1. Protein Synthesis

The present study showed that protein synthesis in the white muscle, liver and whole body of juvenile barramundi exhibited a significant post-prandial increase 4 hours after feeding at temperatures of 27 and 33°C. The increase in protein synthesis after feeding has also been documented in rainbow trout, *Onchorhynchus mykiss* (McMillan and Houlihan, 1989) and Atlantic cod, *Gadus morhua* (Lyndon, *et al.*, 1992). However, even though neither of these studies examined the effect of temperature on post-prandial protein synthesis, these experiments were conducted under optimal temperatures for growth in both species. In the present study, both temperatures (27 and 33°C) were within the optimal temperature range for growth efficiency for barramundi of this size (Chapter 4) with a plateau in growth observed between these temperatures (Katersky and Carter, 2005). The relationships between protein synthesis and growth were evident with no significant differences detected between the synthesis rates for the WM, liver or whole body at any time at 27 and 33°C. In addition, the relationships between feed intake, growth and protein

synthesis rates were clear at 21°C with low feed intake, low growth and no post-prandial effect observed in any tissue over 24 hours.

In all tissues at 27 and 33°C the peak of protein synthesis occurred at 4 h after feeding and by 12 h after feeding protein synthesis had returned to pre-feeding levels with the exception of the liver at 33°C which remained significantly elevated at 12 h. These results differed from previous work on Atlantic Salmon (Fauconneau, *et al.*, 1989) and rainbow trout (McMillan and Houlihan, 1989) where different tissues peaked at different times. The WM k_s peaked at 18 h and 6 h after feeding, respectively and the WB k_s in Atlantic cod peaked 18 h after feeding (Lyndon, *et al.*, 1992). The difference in times of the post-prandial peaks can possibly be attributed to differences between the species, natural environments. Barramundi, a tropical species, inhabits a very different thermal environment to Atlantic salmon, rainbow trout and cod which are all cold-water species. This difference is also compounded by the differences in size between the experimental fish and the feeding regimes under which these experiments were conducted. The barramundi used in the present study were 10 g juveniles while the Atlantic salmon were 43 g (Fauconneau, *et al.*, 1989), the rainbow trout were 50 g (McMillan and Houlihan, 1989) and the cod 177 g (Lyndon, *et al.*, 1992). As the size of the fish increases, the metabolic rate and protein metabolism on a weight-specific basis will decrease (Houlihan, *et al.*, 1995b). The fractional rates of protein synthesis are calculated on per gram of protein basis and therefore would follow the allometry associated with metabolic rate. In addition, the studies on Atlantic salmon, rainbow trout and cod examined the effects of starvation and re-feeding on the protein synthesis (Fauconneau, *et al.*, 1989; McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992). In rainbow trout which were continually fed, Fauconneau *et al.*, (1989) found that protein synthesis was the same at 2 and 18 h after feeding, the two times measured. There fore it is not know whether protein synthesis peaked between these times.

The magnitude of the peak in each tissue is dependant on the feeding regime of the fish. In the present study, fish were fed to satiation daily and the peak rates of liver k_s were 204 and 216 % the pre-feeding rates at 27 and 33°C, respectively. This corresponds well with data from McMillian and Houlihan (1989) on rainbow trout fed daily with liver k_s peaking at ~ 225% the pre-feeding rate within 2 h of feeding (reviewed by Carter and Houlihan, 2001). Both studies (McMillian and Houlihan, 1989; this study) were on a stable nutritional plane and show small peaks in liver k_s in comparison to cod, which were starved for 6 d and re-fed, with peaks of over 1500% the pre-feeding rate (Lyndon et al., 1992; reviewed by Carter and Houlihan, 2001). It seems apparent that the height of the peak is due to a compensatory response to refeeding (Carter, *et al.*, 2001). The large influx of dietary protein from refeeding stimulates protein synthesis in the liver and this sharp increase in protein synthesis may be a mechanism to regulate the influx of amino acids into the free pool (reviewed by Carter and Houlihan, 2001).

In the present study, liver protein synthesis was on average at least an order of magnitude greater than the WM protein synthesis at all temperatures. This relationship has been established for both endotherms and ectotherms (reviewed by McCarthy and Houlihan, 1997). The high synthesis rates are reflective of the livers central role in protein metabolism and it has been suggested by Jobling (1981) that the liver protein synthesis may be a major contributor to SDA. In the present study the mean (± s.e.) absolute protein synthesis at the peak of liver protein synthesis (4 h after feeding) was 258.16 ± 61.42 mg protein · d^{-1} at 27°C and 492.03 ± 111.59 mg protein · d^{-1} at 33°C compared to the white muscle which was 13.20 ± 4.46 mg protein · d^{-1} at 27°C and 26.41 ± 6.21 mg protein · d^{-1} at 33°C. Meaning that at the peak of protein synthesis the liver was synthesizing nearly 20 times the absolute amount of protein as the entire white muscle. This strengthens the argument that the initial increase in SDA could be driven by the energetic demands of the liver protein synthesis. Furthermore, the liver protein synthesis remained high at 8 and 12 h after feeding at temperatures within the optimal range for growth efficiency (Chapter 4).

Sustaining these high synthesis rates is energetically demanding and to expend high levels of energy for such a long time and still maintain maximum growth efficiency, the dietary nutrient balance must be optimal (reviewed by Carter and Houlihan, 2001). When dietary nutrients are not balanced or in excess, protein synthesis and ammonia excretion is increased to regulate the increased intake of amino acids, (Carter, *et al.*, 1995) this increase in synthesis does not translate into growth. Proteins are degraded, increasing energy expenditures and decreasing the energy available for growth and therefore whole body protein growth efficiency.

The relationship between the white muscle and whole body protein synthesis is of considerable interest in order to predict WB k_s from the WM k_s. In the present study the r^2 for this relationship is 0.703 indicating that 70% of the WB k_s was explained by the WM k_s. White muscle k_s is considerably easier to measure than the whole fish simply because of sample size. The white muscle is the largest tissue in the fish and therefore the most representative of the whole body protein synthesis rates. Linear relationships exist between WM k_s and WB k_s and have been determined for barramundi (Chapter 2, this study), cod (Houlihan, *et al.*, 1988), rainbow trout (Fauconneau, *et al.*, 1990), and wolffish, *Anarhichas lupus* (McCarthy, *et al.*, 1999). This relationship also illustrates that regardless of species differences the WB k_s is between 2-4 times the WM k_s (reviewed by Carter and Houlihan; this study).

5.5.2. Mechanisms of protein synthesis

It is apparent from the present study that protein synthesis is driven by increases in post-prandial ribosomal activity rather than increases in either the RNA concentration or Cs. These findings are consistent with those of McMillian and Houlihan (1988; 1989) and Lyndon *et al.* (1992) where upon feeding the Cs values remained at a stable level but the k_{RNA} was stimulated by feeding and followed similar patterns to protein synthesis over time. This substantiates that feeding stimulates RNA translation (the mechanism of protein synthesis), thus enabling synthesis to occur at

an increased rate while the concentrations of RNA and its relationship with protein (Cs) remain relatively stable over the 24 h period. Short-term starvation does not affect the RNA concentration (McMillan and Houlihan, 1989), it takes ~ 2 weeks for the RNA concentration to be altered by ration level (Houlihan, *et al.*, 1993), when refeeding occurs it is the ribosomal activity that enables synthesis to occur at high levels not the concentration of RNA (Lyndon, *et al.*, 1992). Temperature also affects the k_{RNA} and its influence on protein synthesis (Foster, *et al.*, 1992). This is apparent when examining the liver, where the effect of temperature and time have been separated (Fig 5.5) the temperature effect alone significantly increases the k_{RNA} whereas the changes in k_{RNA} with time followed the same pattern as protein synthesis with increases at 4, 8 and 12 h after feeding. Linear relationships have been shown to exist between temperature and k_{RNA} in tissues. Interestingly, the k_{RNA} were not different between tissues, it appears that the k_{RNA} elicit a single linear temperature response (reviewed by McCarthy and Houlihan, 1997). Therefore, the magnitude of the protein synthesis rate is dependant upon the absolute amount of the RNA: protein. The liver Cs values were approximately an order of magnitude greater than the Cs for the WM or WB, which is directly translated into similar differences seen between the rates of protein synthesis. These relationships seem to be constant irrespective of species and have been seen in cod (Lyndon, *et al.*, 1992), rainbow trout (McMillan and Houlihan, 1988; 1989), European eel (de la Higuera, *et al.*, 1999) and rats (Millward, *et al.*, 1972; Waterlow, 2006).

5.6. REFERENCES

Ashford, A.J., Pain, V.M., 1985. Effects of diabetes in the rates of synthesis and degradation of ribosomes in rat muscle and liver *in vivo*. Journal of Biological Chemistry 261, 4059-4065.

Boonyaratpalin, M., Williams, K., 2002. Asian Sea Bass, *Lates calcarifer*. In: Webster, C.D., C., L. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, pp. 40-50.

Brett, J.R., Groves, T.D.D., 1979. Physiological Energetics. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Vol. VIII: Bioenergetics and Growth. Acedemic Press, New York, NY, pp. 279-351.

Brown, M.E., 1957. Experimental studies on growth. In: Brown, M.E. (Ed.), The Physiology of Fishes Vol. I. Academic Press, New York, pp. 361-400.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Wright, P., Anderson, P. (Eds.), Fish Physiology, Vol. 20: Nitrogen Excretion. Academic Press, New York, NY, pp. 31-75.

Carter, C.G., He, Z.Y., Houlihan, D.F., McCarthy, I.D., Davidson, I., 1995. Effect of feeding on tissue free amino acid concentrations in rainbow trout (*Onchorynchus mykiss* Walbaum). Fish Physiology and Biochemistry 14, 153-164.

Carter, C.G., Houlihan, D.F., Kiessling, A., Medale, F., Jobling, M., 2001. Physiological effects of feeding. In: Houlihan, D.F., Boujard, T., Jobling, M. (Eds.), Food Intake in Fishes. Blackwell Scientific, Oxford, pp. 297-331.

de la Higuera, M., Akharbach, H., Hidalgo, M.C., Peragon, J., Lupianez, J.A., Garcia-Gallego, M., 1999. Liver and white muscle protein turnover rates in the European eel (*Anguilla anguilla*): effects of dietary protein quality. Aquaculture 179, 203-216.

Elliott, J.M., 1994. Growth and energetics of brown trout. In: Elliott, J.M. (Ed.), Quantitative ecology and the brown trout. Oxford University Press, Oxford, pp. 69-102.

Fauconneau, B., Breque, J., Bielle, C., 1989. Influence of feeding on protein metabolism in Atlantic Salmon (*Salmo salar*). Aquaculture 79, 29-36.

Fauconneau, B., Aguirre, P., Blanc, J.M., 1990. Protein synthesis in different tissues of mature rainbow trout (*Salmo gairdneri* R.). Influence of triploidy. Comparative Biochemistry and Physiology Part C 97, 345-352.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod. Canadian Journal of Zoology 70, 2095-2102.

Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissue by injection of [^{3}H] phenylalanine. Biochemical Journal 192, 719-723.

Houlihan, D.F., 1991. Protein turnover in ectotherms and its relationship to energetics. In: Gilled, R. (Ed.), Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin Heidelberg, pp. 1-43.

Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effects of body size. Physiological Zoology 59, 482-493.

Houlihan, D.F., Mathers, E.M., Foster, A., 1993. Biochemical correlates of growth rate in fish. In: Rankin, J.C.a.J., F.B. (Ed.), Fish Ecophysiology. Chapman and Hall, London, pp. 45-68.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995a. Protein turnover in animals. In: Walsh, P.J., Wright, P. (Eds.), Nitrogen metabolism and excretion. CRC Press, New York, pp. 1-32.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45, 951-964.

Houlihan, D.F., McCarthy, I., Carter, C.G., Marttin, F., 1995b. Protein turnover and amino acid flux in fish larvae, ICES Marine Science Symposium, pp. 87-99.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. Journal of Fish Biology 18, 385-400.

Jobling, M., 1983. Towards an explanation of specific dynamic action (SDA). Journal of Fish Biology 23, 529-555.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Katersky, R.S., Carter, C.G., 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. Journal of Comparative Physiology B 162, 209-215.

McCarthy, I., Houlihan, D.F., 1997. The effect of temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (*Salmo salar* L.) stocks in Europe as a result of global warming. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 51-77.

McCarthy, I.D., Moksness, E., Pavlov, D.A., Houlihan, D.F., 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Sciences 56, 231-241.

McCue, M.D., 2006. Specific dynamic action: A century of investigation. Comparative Biochemistry and Physiology - Part A 144, 381-394.

McMillan, D.N., Houlihan, D., 1988. The effect of refeeding on tissues protein synthesis in rainbow trout. Physiological Zoology 61, 429-441.

McMillan, D.N., Houlihan, D.F., 1989. Short-term responses of protein synthesis to re-feeding in rainbow trout. Aquaculture 79, 37-46.

Millward, D.J., Garlick, P., James, W.P.T., Nnanyflugo, D.O., Ryatt, J.S., 1972. Relationship between protein synthesis and RNA content in skeletal muscle. Nature 241, 204-205.

Sugden, P.H., Fuller, S.J., 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochemical Journal 273, 21-37.

Taylor, P.M., Brameld, J.M., 1999. Mechanisms and regulation of transcription and translation. In: Lobley, G.E., White, A., MacRae, J.C. (Eds.), Protein metabolism and nutrition. Wageningen Pers, Wageningen, pp. 25-50.

Waterlow, J.C., 2006. Protein turnover in tissues: Effects of food and hormones. In: Waterlow, J.C. (Ed.), Protein turnover. CAB International, Oxfordshire, pp. 223-249.

CHAPTER 6

THE EFFECT OF HIGH TEMPERAUTRES ON FEED INTAKE AND PROTEIN SYNTHESIS OF JUVENILE BARRAMUNDI, *LATES CALCARIFER*

6.1. ABSTRACT

The aim of this experiment was to measure the post-prandial protein synthesis at temperatures which encompassed and exceeded the optimal temperature range for growth efficiency as well as incorporate the data from the preceding growth trial (Chapter 3) in order to calculate measures of protein turnover. Juvenile barramundi (4.87 ± 0.32 g) were held at 27, 33, 26 and 39°C and fed to satiation twice daily (503.5 $g \cdot kg^{-1}$ crude protein, 182.5. $g \cdot kg^{-1}$ lipid, 150.1 $g \cdot kg^{-1}$ ash, 20.5 GE $MJ \cdot kg^{-1}$). Samples were taken over a 24 h period at time 0 (24 h after the previous meal) and then 2, 4, 6, 8 12 and 24 h after feeding to measure protein synthesis in the white muscle, liver, stomach, digestive tract and whole body. Validation of the flooding dose was not confirmed for the liver, stomach and the digestive tract. Feed intake on the day of the flooding dose was low compared with the preceding growth trial (Chapter 3) and previous protein synthesis work (Chapter 5) and this resulted in low protein synthesis values and a depressed post-prandial event for both the white muscle and whole body. However, a post-prandial increase in protein synthesis was evident at 2 and 4 h after feeding in the whole body at 27 and 33°C. There was a significant negative relationship between the anabolic stimulation efficiency and protein growth. When data were analyzed by temperature, it was clear that at 39°C where high anabolic stimulation occurred there was low growth indicating an increase in protein turnover at high temperatures. The RNA:Protein was similar to previously determined values (Chapter 5). Suggesting that protein synthesis in the present study had the capacity to attain similar levels as found previously (Chapter 5). Low feed intake was identified as the key limiting factor that determined rates of protein synthesis.

Keywords: Feed intake; *Lates calcarifer*; Protein synthesis; SDA; Temperature

6.2. INTRODUCTION

The influence of feed intake on the physiology of fish has been extensively reviewed (Jobling, 1983; Millward, 1989; Houlihan, 1991; Houlihan, *et al.*, 1995a; Carter, *et al.*, 2001; McCue, 2006). It is generally accepted that increased feed intake will increase growth in fish through increases in protein synthesis when food is unlimited and environmental conditions are optimal (reviewed by Carter, *et al.*, 2001). Protein synthesis is the underlying factor driving growth of all animals. It is an energetically expensive process which has been shown to account for up to 42% of the total energy expenditures (reviewed by Carter and Houlihan, 2001). It is therefore critical in order to maintain high growth efficiency that the nutrient balance in the diet is ideal. Increasing the protein:energy above a critical level for a species will result in additional energy expenditures to process dietary amino acids which will only be excreted and consequently lower the energy available for growth and the efficiency at which it occurs (Carter and Houlihan, 2001). After feeding, there is an increase in metabolic rate, variously known as heat increment or specific dynamic action (SDA) and which encompasses all physiological processes involved in nutrient processing, including protein synthesis (reviewed by McCue, 2006). The increase in protein synthesis occurs to regulate the dietary amino acid influx, maintain relatively stable free pool amino acids concentrations and maximize growth (Houlihan, *et al.*, 1995b).

SDA is easily affected by a number of endogenous and exogenous factors (reviewed by Jobling, 1983; McCue, 2006). It is without question that temperature is a key environmental parameter influencing the physiology of fish. As temperature increases, feed intake will increase until the temperature raises above the optimal temperature range for growth efficiency (Chapter 4), at this point feed intake decreases as the temperature continues towards the upper thermal limit. Increased feed intake potentially causes a larger SDA event to occur (McCue, 2006). It is proposed that the energetic costs associated with protein synthesis are the driving force behind the rapid increase in metabolism after feeding (Jobling, 1983).

The relationship between temperature, feed intake, growth and protein synthesis are dependant upon one another and are often expressed through a series of linear regressions (Houlihan, *et al.*, 1993; Houlihan, *et al.*, 1995a). This was explored in Atlantic cod, *Gadus morhua* which were held at two different temperatures, 5 and 15°C (Foster, *et al.*, 1992). When fish were fed a similar ration at different temperatures no difference in protein synthesis was apparent between the fish (Foster, *et al.*, 1992), however when fish held at different temperatures are fed *ad libitum* rations a significant increase in protein synthesis occurs (reviewed by McCarthy and Houlihan, 1997). Therefore, protein synthesis is dependant upon feed intake irrespective of temperature.

The mechanism of protein synthesis is RNA translation (Taylor and Brameld, 1999). The relationships between the concentration of RNA, RNA:Protein and the RNA activity have received attention in determining the underlying processes of protein synthesis at different temperatures (reviewed by McCarthy and Houlihan, 1997). It is the current view that after feed intake, the RNA concentration, expressed as $\mu g \cdot mg^{-1}$ or its ratio to protein, remains relatively stable after feed intake (McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992) and that ribosomal activity drives protein synthesis (McMillan and Houlihan, 1989; Foster, *et al.*, 1992; Lyndon, *et al.*, 1992). When RNA activity is examined after feeding, it necessarily follows the same patterns as protein synthesis with peaks of activity occurring at peak times of protein synthesis (Chapter 5). Differences in RNA concentration will affect the relative protein synthesis in different tissues with the RNA concentration in the liver being an order of magnitude greater than the RNA concentration in the white muscle and these differences being directly translated to differences observed in protein synthesis (Lyndon, *et al.*, 1992; Chapter 5).

Barramundi, *Lates calcarifer*, have a wide thermal tolerance range. The majority of research on protein metabolism in relation to temperature has focused on cold water

species such as rainbow trout, *Onchorynchus mykiss* (Fauconneau and Arnal, 1985), Atlantic Cod, *Gadus morhua* (Foster, *et al.*, 1992), Atlantic salmon *Salmo salar* (reviewed by McCarthy and Houlihan, 1997) and Atlantic wolfish, *Anarhichas lupus* (McCarthy, *et al.*, 1999). This is the first study to examine extreme high temperatures on a tropical species. Barramundi has an optimal temperature range for growth efficiency of 25-37°C (Chapter 4). Having such a wide range of temperatures where maximum growth efficiency occurs provides an interesting opportunity to examine how fish cope at different temperatures and metabolic rate to maintain efficiency. The purpose of this experiment was to examine the protein metabolism at temperatures across the optimal temperature range as well as at a temperature which exceed this range and approached the upper thermal limits of the species.

This study was conducted in order to continue the temperature profiling of protein metabolism that was started in Chapter 5. Unfortunately, in the present study, on the day of the flooding dose the feed intake was low and this was reflected in lower protein synthesis of both the white muscle and the whole body. An attempt has been made to correct for the low feed intake by predicting the protein growth that would have occurred from protien feed intake on the day of the protein synthesis measurement. These calculations are based upon the feed intake and growth from the preceding growth trial (Chapter 3) which uses the same fish. Chapter 7 presents these data in terms of protein turnover and models of protein metabolism over the entire thermal tolerance range.

6.2. MATERIALS AND METHODS

6.2.1. Experimental Diet

A standard diet was formulated according to known dietary requirements for barramundi (Boonyaratpalin and Williams, 2002). The diet (Table 6.1) was formulated to contain 50% crude protein and 19.7 MJ kg^{-1} gross energy (Katersky and Carter, 2005). Fish meal and fish oil were supplied by Skretting (Tasmania,

Australia). Vitamins and minerals were supplied by Sigma-Aldrich Pty. Ltd (Sydney, Australia), Vitamin C was supplied as Stay-C from Roche Pharmaceuticals (Roche Vitamins Australia Ltd., Sydney, Australia).

6.2.2. Acclimation Period

Juvenile barramundi (1 g) were obtained from WBA Hatcheries (South Australia, Australia). Fish were maintained at the University of Tasmania under constant environmental conditions (salinity: 10 ‰; photoperiod: 24h light (Barlow, 1995); temperature: 27°C). Fish were initially stocked into 4 150-l aquaria and maintained at 27°C. Temperature was adjusted 1°C d^{-1} towards the experimental temperatures of 33, 36 and 39°C, with the exception of the 27°C aquarium which was maintained at a constant temperature. After 12 days all fish were at their experimental temperature. The experiment was conducted in four identical recirculating systems each consisting of 3 19-l carboys with a trickle biofilters on each system (Katersky and Carter, 2005). Each system was held at a constant temperature (either 27, 33, 36 or 39°C) with submersible heaters each controlled with an individual thermostat. All fish were fed the standard diet to satiation twice daily during the preceding growth trial. The flooding dose experiment was conducted at the end of a 20 d growth trial (Chapter 3).

6.2.4. Protein Synthesis

Samples were taken at time 0 (24 h after the previous meal) and then 2, 4, 6, 8, 12 and 24 h after feeding in order to measure rates of protein synthesis in the white muscle (WM), liver, stomach, digestive tract and whole fish (WB). At each time, 2 fish were sampled from each replicate tank per treatment, one for whole body and the other for organ analysis.

Rates of protein synthesis were measured following a single injection of ^{3}H-phenylalanine using the flooding-dose method (Garlick, *et al.*, 1980; Houlihan, *et al.*,

1986). At specific times (0, 2, 4, 6, 8, 12 and 24 h) after their final meal, barramundi were anesthetized with benzocaine (100 $mg \cdot L^{-1}$), weighed and injected into the caudal vein with ^{3}H-phenylalanine at a concentration of $1ml \cdot 100g$ body $weight^{-1}$. The injection solution contained150 μmol L-phenylalanine and L-[2,6-^{3}H]phenylalanine (Amersham Pharmacia Biotech, NSW, Australia) in 0.2 μm filtered seawater at pH 7.4. The measured specific activity of the injection solutions were 1123 ± 118 dpm $nmol^{-1}$ phenylalanine.

Following injection fish were returned to separate aquaria containing aerated water (10‰) incorporation times of ~40-60 min were used as determined from preliminary experiments to establish incorporation times at high temperatures (Katersky, unpublished data). Following incorporation, fish were removed from the tank, killed by an overdose of benzocaine (400 $mg \cdot L^{-1}$) and transection of the spinal cord. Individual samples of WM, liver and the remaining carcass from each temperature treatment were frozen in liquid nitrogen for later analysis. The subsequent analysis of samples to measure protein-bound and free-pool phenylalanine-specific radioactivities was as described previously (Houlihan, *et al.*, 1988; Houlihan, *et al.*, 1995b). Briefly, protein concentrations were measured using a modification of the folin-phenol method (Lowry, *et al.*, 1951) and RNA concentrations ([RNA]) were measured using dual wavelength absorbance (Ashford and Pain, 1985). RNA was also expressed as the capacity for protein synthesis (Cs: mg RNA· g $protein^{-1}$) and as RNA activity (k_{RNA}, $k_s \cdot g^{-1}$ $RNA \cdot d^{-1}$) (Sugden and Fuller, 1991).

Fractional rates of protein synthesis for WM, liver and carcass (k_s, $\% \cdot d^{-1}$) were calculated according to the following formula:

$$k_s = (S_b/S_a) \cdot (1440/t) \cdot 100 \quad (1)$$

where S_b is the protein bound phenylalanine specific radioactivity; S_a is the free-pool phenylalanine specific radioactivity; t is the post injection incubation time in minutes,

(Garlick, et al., 1980). WBk_s was calculated using the mean WMs_a value for each temperature, no significant differences were found among the WMs_a values over the course of the day within each temperature treatment (27°C: F=0.83; df=6,19; p=0.57; 33°C: F=1.47; df=6,21; p=0.26; 36°C: F=0.48; df=6,21; p=0.81; 39°C: F=1.03; df=6,21; p=0.45).

6.2.5. Protein Turnover

Daily rates of protein synthesis were determined by the proportional mean WBk_s at each sample period. Protein degradation was then calculated from the difference between average daily rates of protein accretion and protein synthesis. Synthesis retention efficiency (SRE, %) was determined by dividing k_g by k_s (Houlihan, *et al.*, 1995a). Calculations for protein turnover were done according to the formulae used by Houlihan et al., (1995). Data on feed intake, growth, productive protein value (PPV) and body composition were obtained from Chapter 3.

6.2.6. Statistical Analysis

Data are presented as mean ± standard error. The normality and homogeneity of data were explored by examining the residual plots. Protein synthesis and measures of RNA results were analyzed using a two-way ANOVA (SPSS, version 11.5) to look at the effects of temperature and the time after feeding. Where there was a significant interaction a one-way ANOVA followed by a Tukey HSD were used to identify significantly different means. When interactions of these two factors were not significant the individual 1-way ANOVA results were examined and significant results were compared using Tukey's HSD. Measures of protein turnover were analyzed using a one-way ANOVA and significant results were compared using Tukey's HSD.

Table 6.1. Ingredient and chemical composition of experimental diet.

Ingreadient Compostion ($g \cdot kg^{-1}$)	
Fish meal	730
Fish oil	70
Starch	119
CMC	10
Choline chloride	10
Phosphorus ($NaPO_4$)	10
Vitamin C (Stay-C)	20
Yetterbium-oxide	1
Vitamin premix[a]	15
Mineral premix[b]	15
Chemical Compostion ($g \cdot kg^{-1}$ DM)	
Dry matter ($g \cdot kg^{-1}$)	946.9
Crude protein	503.5
Crude lipid	182.5
Ash	150.1
Energy ($MJ \cdot kg^{-1}$)	20.52

[a]Vitamin premix (mg kg^{-1}): Vitamin A (7.50), Vitamin D (9.00), Rovimix E50 (150.00), Menadione sodium bisulphate (3.00), Riboflavin (6.00), Calcium D-pantothenate (32.68), Nicontinic Acid (15.00), Vitamin B-12 (0.015), d-biotin (0.23), Folic acid (1.50), Thiamin HCl (1.68), Pyridoxine HCl (5.49), myo-Inositol (450.00), α-cellulose (817.91).

[b]Mineral premix (mg kg^{-1}): $CuSO_4$ $5H_20$ (35.37), $FeSO_4$ $7H_2O$ (544.65), $MnSO_4$ H_2O (92.28), Na_2SeO_3 (0.99), $ZnSO_4$ $7H_2O$ (197.91), KI (2.16), $CoSO_4$ $7H_2O$ (14.31), α-cellulose (612.33).

6.3. RESULTS

6.3.1. Feed Intake

Feed intake was significantly lower on the day of the flooding dose for all temperatures except for the 27°C fish when compared to the growth trial described in Chapter 3 (Table 2). This resulted in a 'low ration' being consumed by all fish even though the fish were fed to apparent satiation. To correct for this, protein growth was calculated from the protein consumption on the day of the flooding dose from the relationship between k_c and k_g which was determined from the preceding growth trial (Chapter 3). Feed intake was checked at the time of dissection by visual inspection of the stomach at each sample time, however at times beyond 4 h after feeding it was difficult to tell weather the fish consumed a meal, this was attributed to high gut evacuation rates at high temperatures, although not quantified. All fish sampled at 2 and 4 h after feeding had fed.

6.3.2. Protein Synthesis

6.3.2.1. White Muscle

Validation of the flooding dose method for WM was confirmed from the relationship between the in vivo incubation times and the bound and free pools of ^{3}H-phenylalanine (dpm·nmol^{-1} phenylalanine). A significant positive linear relationship ($p<0.001$; df=1,81; $r^2=0.19$, Fig. 6.1a) between the bound ^{3}H-phenylalanine (s_b) and *in vivo* incubation time (T) was described by the equation:

$$S_b = 0.035T - 1.036 \quad (2)$$

The ^{3}H-phenylalanine free pool (s_a) did not vary over the *in vivo* incubation period ($p=0.906$; df=1,81; $r^2=-0.012$, Fig 6.1b). There was no significant interaction

between temperature and the time after feeding for WMk_s ($F=0.92$; $df=18,81$; $p=0.56$, Fig.6.2), however, a significant effect of both temperature ($F=19.76$; $df=3,81$; $p<0.001$) and time ($F=7.72$; $df=6,81$; $p<0.001$) were present when examined separately. WMk_s significantly decreased with increasing temperature (Fig. 6.3a), the 27°C fish had k_s values over 4 times those of the 39°C fish. There was a significant increase (30%) in WMk_s between 0 and 2 hours after feeding (Fig. 6.3b).

6.3.2.2. Liver, Stomach and Digestive Tract

Validation of the flooding dose method for the liver, stomach and digestive tract was not confirmed from the relationships between the *in vivo* incubation times and the bound and free pools of ^{3}H-phenylalanine (dpm·nmol^{-1} phenylalanine). The relationship between the bound ^{3}H-phenylalanine and *in vivo* incubation time for the liver failed to produce a positive significant linear relationship ($p=0.125$; $df=1,73$; $r^2=0.019$), however, the ^{3}H-phenylalanine free pool had a non-significant relationship ($p=0.371$; $df=1,73$; $r^2=-0.003$) over the *in vivo* incubation period. The relationship between the stomach bound ^{3}H-phenylalanine and the *in vivo* incubation time had a positive significant linear relationship ($p=0.001$; $df=1,80$; $r^2=0.111$), however the ^{3}H-phenylalanine free pool significantly decreased over the incubation period ($p=0.024$; $df=1,73$; $r^2=0.051$). The relationship between the digestive tract bound ^{3}H-phenylalanine and the *in vivo* incubation time failed to produce had a positive significant linear relationship ($p=0.528$; $df=1,37$; $r^2=-0.016$), and the ^{3}H-phenylalanine free pool significantly decreased over the incubation period ($p=0.032$; $df=1,76$; $r^2=0.047$). Furthermore, 45% of the digestive tract free pools had been depleted to less than 25% of the original injection solution. For these reasons, the data were not presented here.

6.3.2.3. Whole Body

There was no significant interaction (F=1.254; df=18,81; p=0.255) between temperature and the time after feeding for WBk_s. However even though this was non significant, an increase in WBk_s for 27 and 33°C fish between 2 and 4 h after feeding was ~ 2 times greater than the WBk_s at 39°C (Fig. 6.4). This increase was significant when the time after feeding was examined separately from temperature (F=8.24; df=6,81; p<0.001) with significant increases occurring between 2 and 4 hours after feeding, by 6 h after feeding the WBk_s was back to pre-feeding levels (Fig. 6.5b). There was a significantly effect of temperature on the WBk_s (F=17.44; df=3,81; p<0.001), the 39°C fish had significantly lower rates of protein synthesis (Fig. 6.5a).

6.3.3. RNA Correlates

6.3.3.1. White Muscle

There was a significant interaction between temperature and the time after feeding for Cs (RNA:Protein) in the WM (F=1.80; df=18,82; p=0.049). Fish at 36 and 39 °C did not show a change in Cs over the 24 h period (Fig 6.6). There was no significant interaction between k_{RNA} and the temperature and time after feeding (F=0.99; df=18,82; p=0.488), however when the factors were examined individually, the time after feeding had a significant effect on k_{RNA} (F=7.07; df=6,82; p<0.001). The pattern of k_{RNA} over the 24 h after feeding was similar to that of WMk_s. There was a significant increase in activity at 2 h after feeding (Fig.6.7). There was no significant effect of temperature on k_{RNA} (F=2.06;df=3,82; p=0.116). There was no significant interaction between temperature and the time after feeding for the concentration of RNA (F=0.52; df=18,82; p=0.938), however when examined individually, there was a significant effect of temperature on the RNA concentration (F=17.34; df=3,82; p<0.001) with the 39°C fish having lower RNA than the other fish (Fig.8). There was

no significant effect of the time after feeding on the concentration of RNA in the WM ($F=1.18$; $df=6,82$; $p=0.332$).

6.3.3.2. Whole Body

There was no significant interaction ($F=1.02$; $df=18,82$; $p=0.456$) between temperature and the time after feeding for Cs. However, temperature has a significant effect on Cs ($F=32.84$; $df=3,82$; $p<0.001$) with the 27°C fish having the highest Cs and 39°C having a significantly lower ratio of RNA and protein than any other temperature treatment (Fig. 6.9). The time after feeding did not have an effect on Cs ($F=0.49$; $df=6,82$; $p=0.814$). There was no significant interaction between temperature and the time after feeding for k_{RNA} ($F=0.91$; $df=18,82$; $p=0.571$). Both temperature ($F=8.61$; $df=3,82$; $p<0.001$) and time ($F=6.90$; $df=3,82$; $p<0.001$) had a significant effect on k_{RNA} when examined individually. At 2 and 4 h after feeding there was a significant increase in RNA activity (Fig. 6.10a), at 6 h after feeding k_{RNA} had returned to pre-feeding levels. At 33°C, k_{RNA} is significantly higher than at 27, 36 or 39°C (Fig. 6.10b). There was a significant interaction between temperature and the time after feeding on the concentration of RNA ($F=1.91$; $df=18,82$; $p=0.035$). The concentration of RNA did not significantly change over 24 h for 33 and 39°C fish (Fig. 6.11).

6.3.4. Protein Turnover

A relationship between k_c and k_g (Fig. 6.12a) from the growth trial (Chapter 3) was established in order to predict k_g on the day of the flooding dose based on the daily feed intake (Fig. 6.12b). There was a significant effect of temperature on the predicted growth rates ($F=4.62$, $df = 8,3$, $p = 0.037$) with the 27, 33 and 36°C fish not having significantly different growth rate but growth at 27°C was higher than at 39°C (Table 6.2). There was a significant positive relationship between k_c and the WMk_s ($WMk_s = 0.25\ kc+0.17$; $F = 16.28$, $r^2 = 0.62$, $df = 11$, $p = 0.002$) and WB k_s

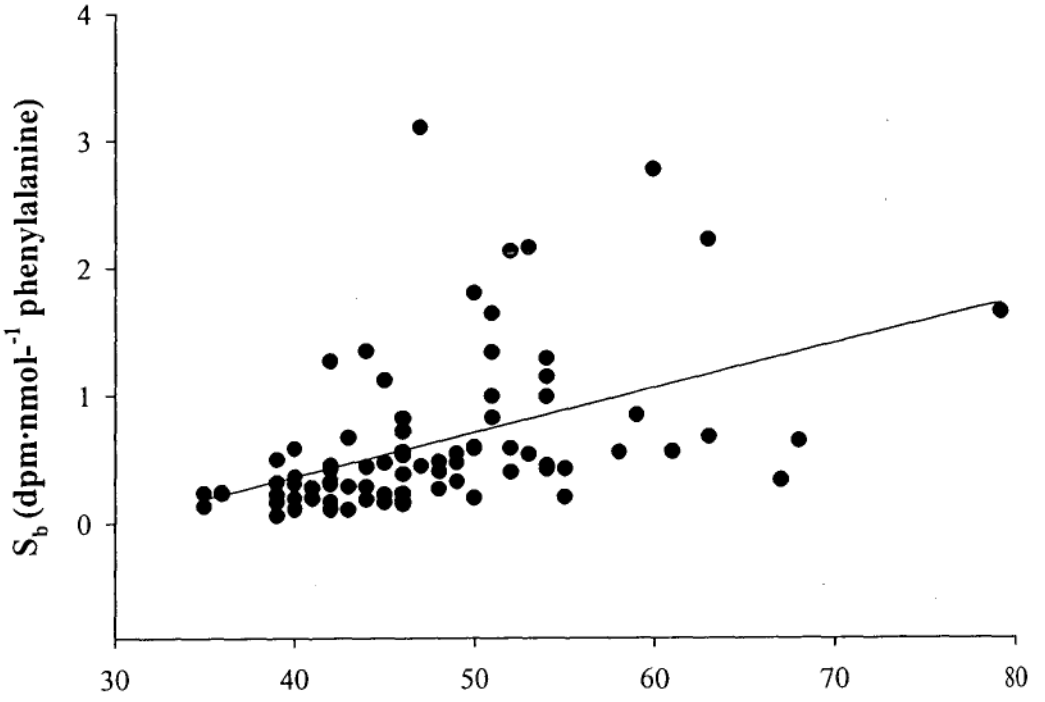
($WBk_s = 0.21k_c+2.70$; $F = 7.76$, $r^2 = 0.44$, $df = 11$, $p = 0.02$) across all temperatures (Fig. 6.12c). The relationship between growth efficiency and SRE was significant ($F = 6.75$, $r^2 = 0.40$, $p = 0.02$) indicating that for higher growth efficiency to occur, the fish would have to retain a higher proportion of proteins (Fig. 6.13a). The SRE was also significantly different between temperatures ($F = 5.13$, df 8,3, $P = 0.029$) with a differences occurring between the 27 and 39°C fish (Table 6.2). There was a significant negative relationship between ASE and the predicted growth ($F = 24.21$, $r^2 = 0.71$, $p<0.001$). With the increased stimulation of proteins there was lower predicted growth, this would have been a function of increased protein turnover (Fig. 6.13b). ASE was not significantly affected by different temperatures ($F = 2.49$, $df = 8,3$, $p = 0.135$, Table 6.2).

Table 6.2. Mean (± S.E.) feed intake ($g \cdot d^{-1}$) on the day of the flooding dose and for the preceding growth trial (Chapter 3), growth, SRE, ASE and growth efficiency for each temperature tested.

Temperature (°C)	27	33	36	39
Feed Intake (Flooding Dose, $g \cdot d^{-1}$)	4.89 ± 0.88	6.75 ± 1.41	4.63 ± 0.34	1.56 ± 0.54
Feed Intake (Growth Trial, $g \cdot d^{-1}$)*	5.84 ± 0.17	11.11 ± 0.42	10.51 ± 0.97	4.79 ± 0.13
SGR ($\%\cdot d^{-1}$)*	5.38 ± 0.19^{a}	7.10 ± 0.07^{b}	7.11 ± 0.42^{b}	2.84 ± 0.09^{c}
Predicted k_g ($\%\cdot d^{-1}$)	3.06 ± 1.42^{a}	1.65 ± 0.92^{a,b}	0.24 ± 0.06^{a,b}	-1.82 ± 0.95^{b}
SRE (%)	67.78 ± 32.10^{a}	31.35 ± 16.73^{a,b}	6.65 ± 2.01^{a,b}	-63.68 ± 32.94^{b}
ASE (%)	51.96 ± 9.16	69.74 ± 9.05	61.44 ± 4.69	100.55 ± 23.04
Predicted PPV (%)†	29.29 ± 8.40^{a}	19.03 ± 8.08^{a}	3.93 ± 0.90^{a,b}	-79.04 ± 41.03^{b}

Synthesis retention efficiency (SRE, %) = protein growth (k_g) / protein synthesis (k_s) *100
Anabolic stimulation efficiency (ASE, %) = protein synthesis (k_s) / protein intake (k_c) *100
Productive protein value (PPV, %) = protein growth (k_g) / protein intake (k_c) *100†
Means with similar subscripts were not significantly different ($p<0.05$, $n = 3$).
*Data on feed intake, growth and efficiency are also presented in Chapter 3.
†Data calculated from the predicted k_g ($\%\cdot d^{-1}$) and protein intake from the day the protein synthesis measurements were made.

Figure 6.1. The incorporation of (a) the bound phenylalanine (S_b dpm·nmol^{-1} phenylalanine) and (b) the free pool (S_a dpm·nmol^{-1} phenylalanine) over the *in vivo* incubation time for barramundi, *Lates calcarifer* across all temperatures. The relationship between bound phenylalanine and the incubation time was S_b=0.035T-1.036 (r^2=0.193; p< 0.001; n=80). The relationship between the free pool and the incubation time was expressed as, S_a = -0.261T+896.82 (r^2=-0.012; p=0.91; n=80).


4
3
2
1
0
S_b (dpm·nmol^{-1} phenylalanine)
30
40
50
60
70
80

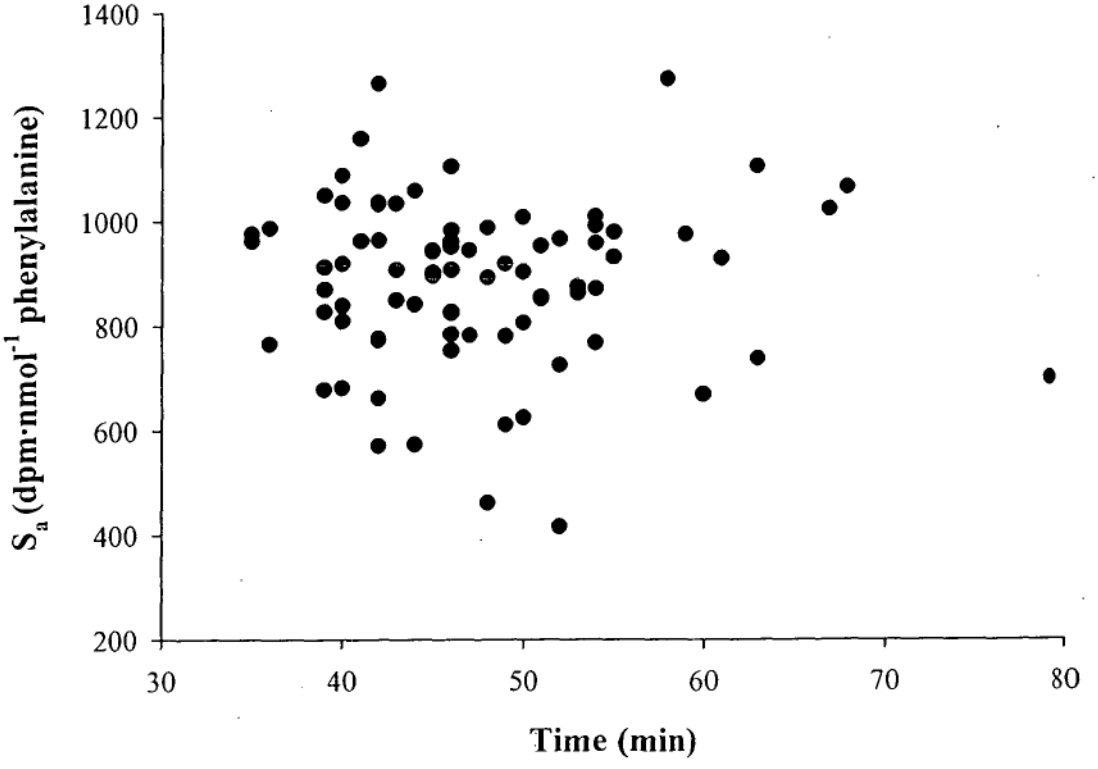

1400
1200
1000
800
600
400
200
S_a (dpm·nmol^{-1} phenylalanine)
30
40
50
60
70
80
Time (min)

Figure 6.2. The effect of feed intake on the fractional rate of protein synthesis (k_s, $\%\cdot d^{-1}$) in (a) white muscle and (b) whole body for juvenile barramundi, *Lates calcarifer*, over 24 h after a final meal at 27, 33, 36 and 39°C.

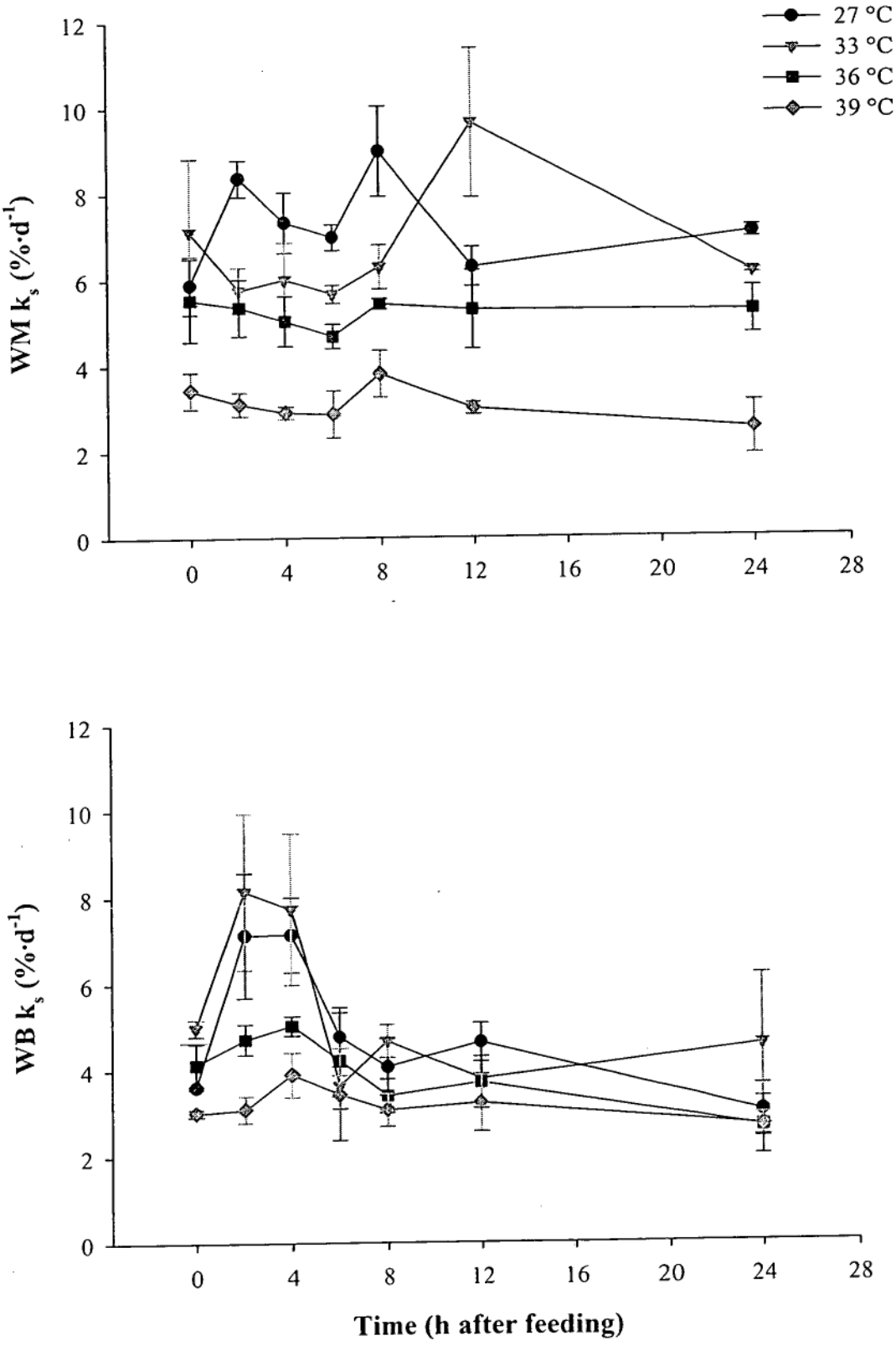
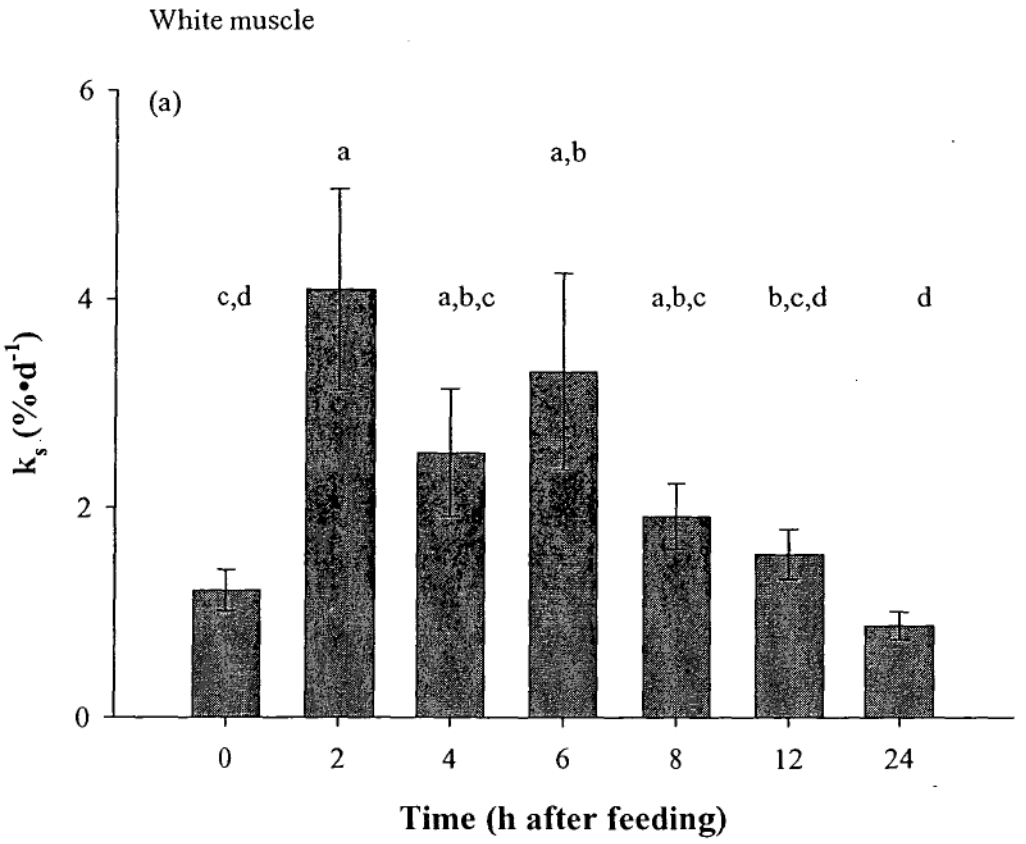

27 °C
33 °C
36 °C
39 °C
WM k_s (%·d^{-1})
WB k_s (%·d^{-1})
0
2
4
6
8
10
12
0
4
8
12
16
20
24
28
Time (h after feeding)

Figure 6.3. The effect of (a) feed intake and (b) temperature on the white muscle protein synthesis (k_s, $\%\cdot d^{-1}$) for juvenile barramundi, *Lates calcarifer*. Letters indicate a significant difference between either the sampling time or temperature ($p<0.05$).


White muscle
(a)
6
4
2
0
k_s (%•d^{-1})
a
a,b
c,d
a,b,c
a,b,c
b,c,d
d
0
2
4
6
8
12
24
Time (h after feeding)

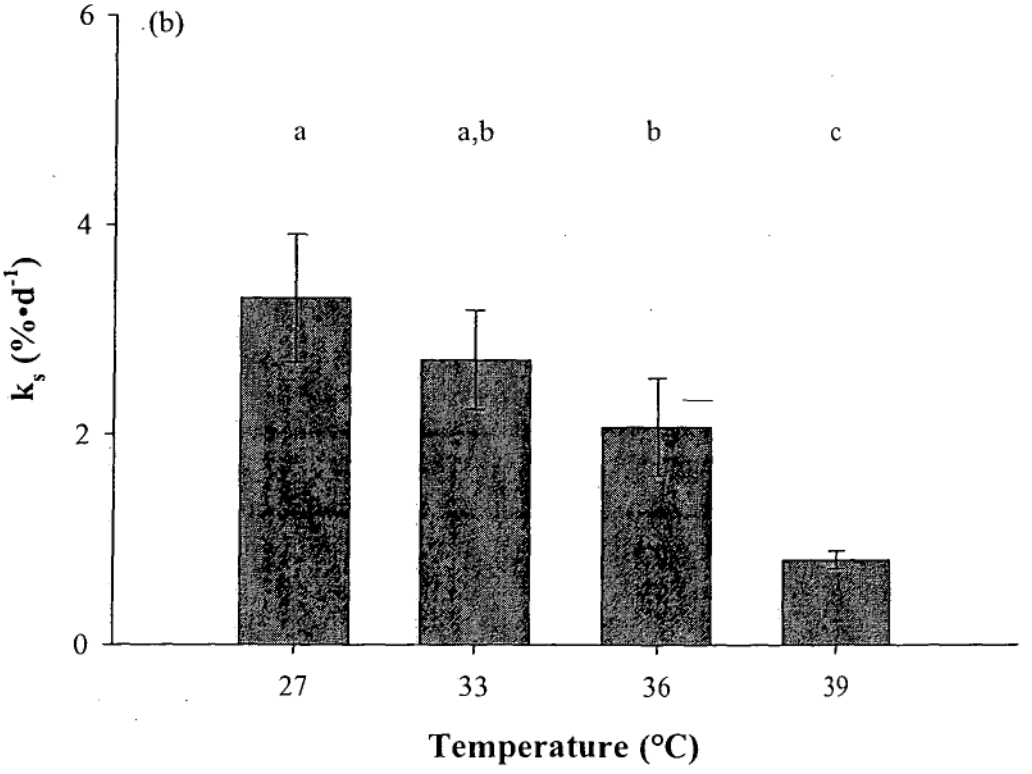
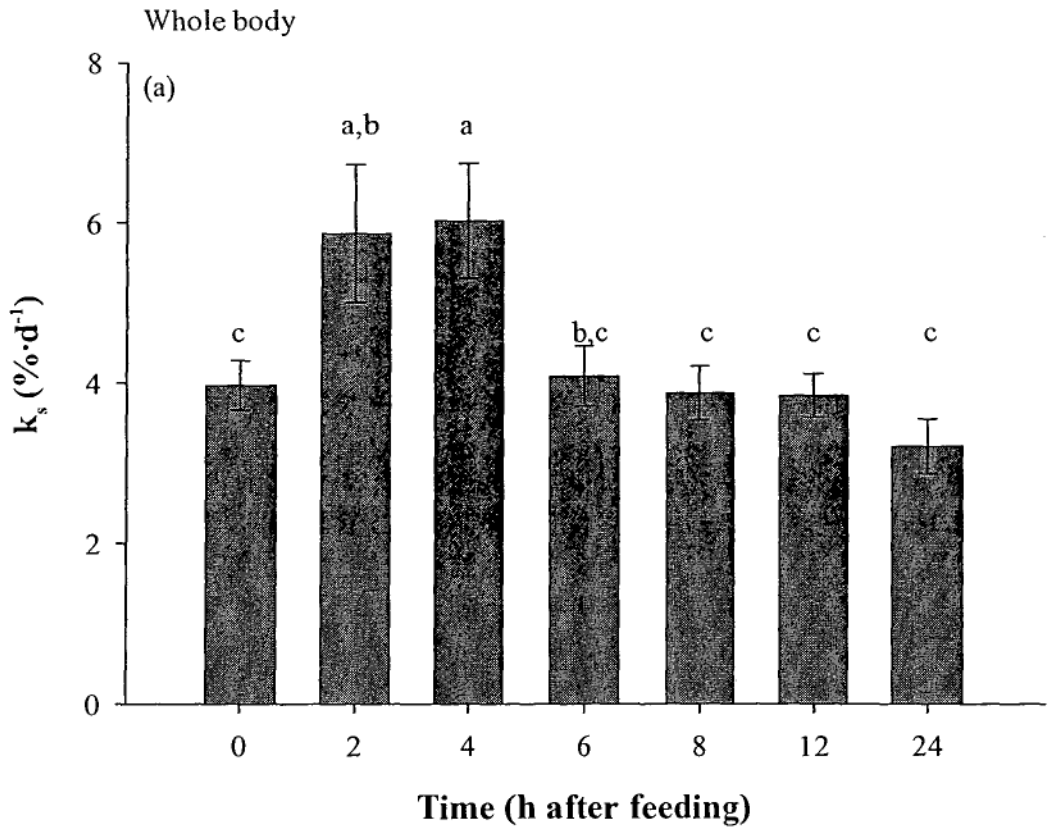

(b)
6
4
2
0
k_s (%•d^{-1})
a
a,b
b
c
27
33
36
39
Temperature (°C)

Figure 6.4. The effect of (a) feed intake and (b) temperature on the whole body protein synthesis (k_s, $\%\cdot d^{-1}$) for juvenile barramundi, *Lates calcarifer*. Letters indicate a significant difference between either the sampling time or temperature ($p<0.05$).


Whole body
(a)
8
6
4
2
0
k_s (%·d^{-1})
a,b
a
c
b,c
c
c
c
0
2
4
6
8
12
24
Time (h after feeding)

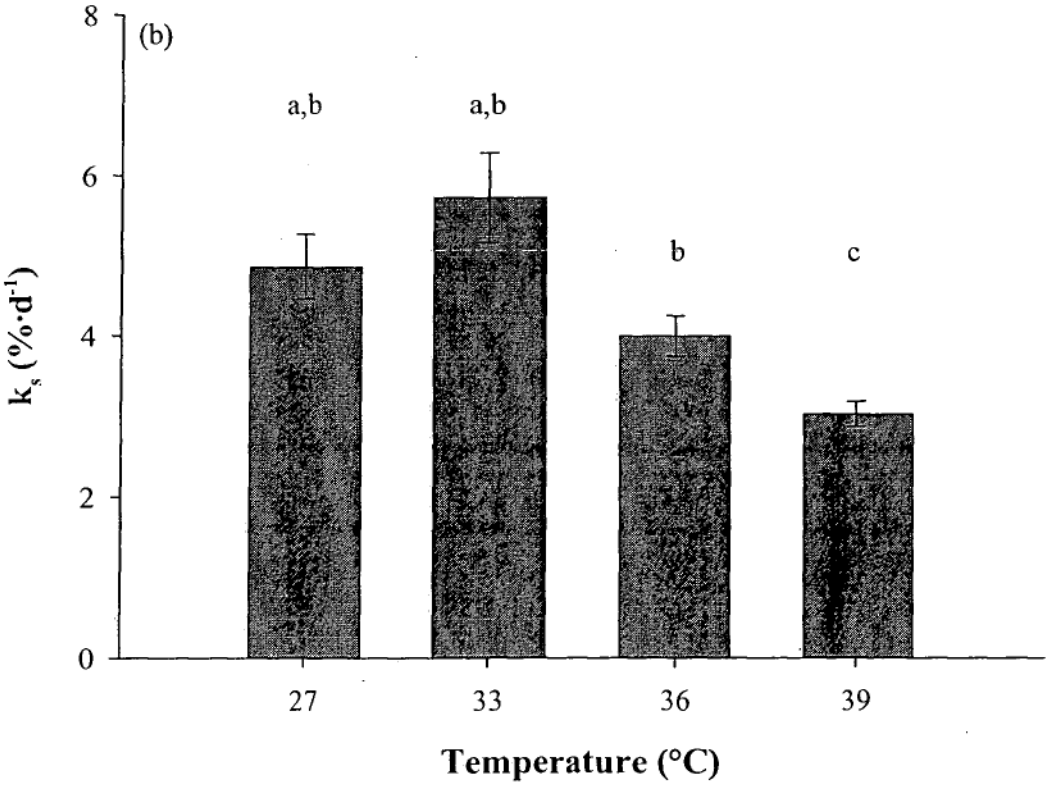
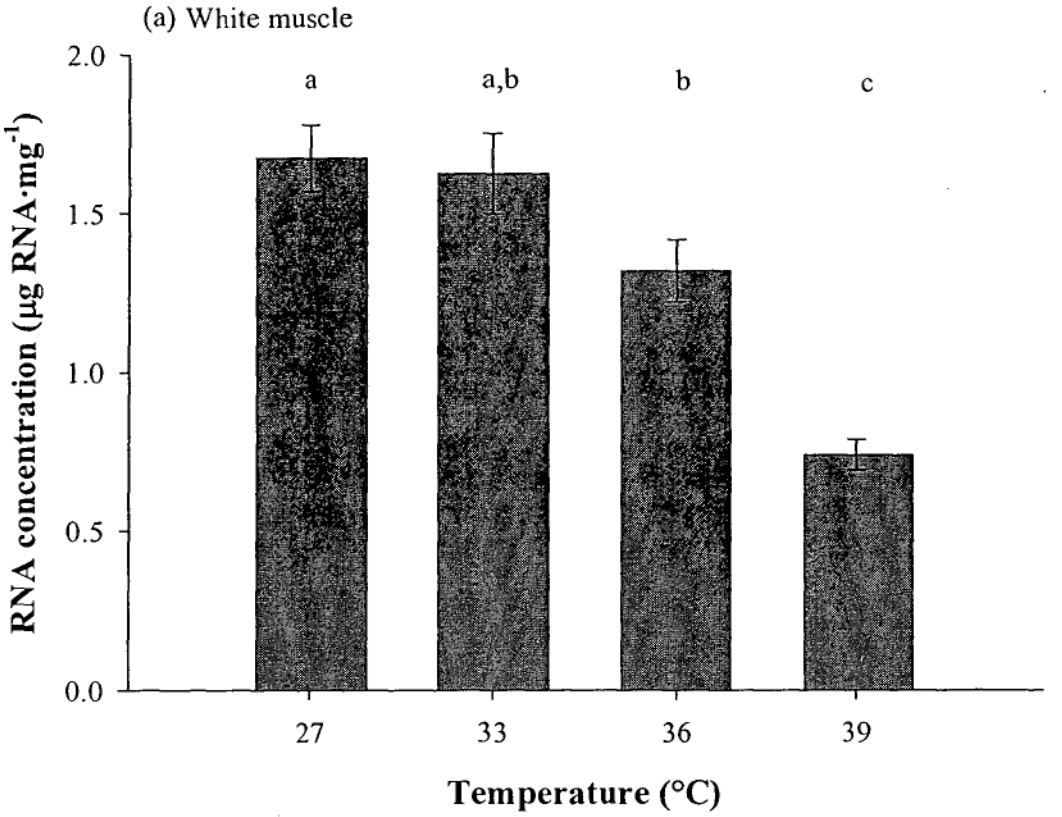

(b)
8
6
4
2
0
k_s (%·d^{-1})
a,b
a,b
b
c
27
33
36
39
Temperature (°C)

Figure 6.5. The effect of temperature on the RNA concentration (μg RNA · mg^{-1}) for (a) white muscle for juvenile barramundi *Lates calcarifer*. Letters indicate a significant difference between temperatures ($p<0.05$). (b) The effect of feed intake on the RNA concentration (μg RNA · mg^{-1}) for whole body over 24 h after a final meal at 27, 33, 36 and 39°C. An asterisk (*) indicate significant 2-way interaction is between the temperature and the time after feeding ($p<0.05$).


(a) White muscle
RNA concentration (µg RNA·mg-1)
2.0
1.5
1.0
0.5
0.0
a
a,b
b
c
27
33
36
39
Temperature (°C)

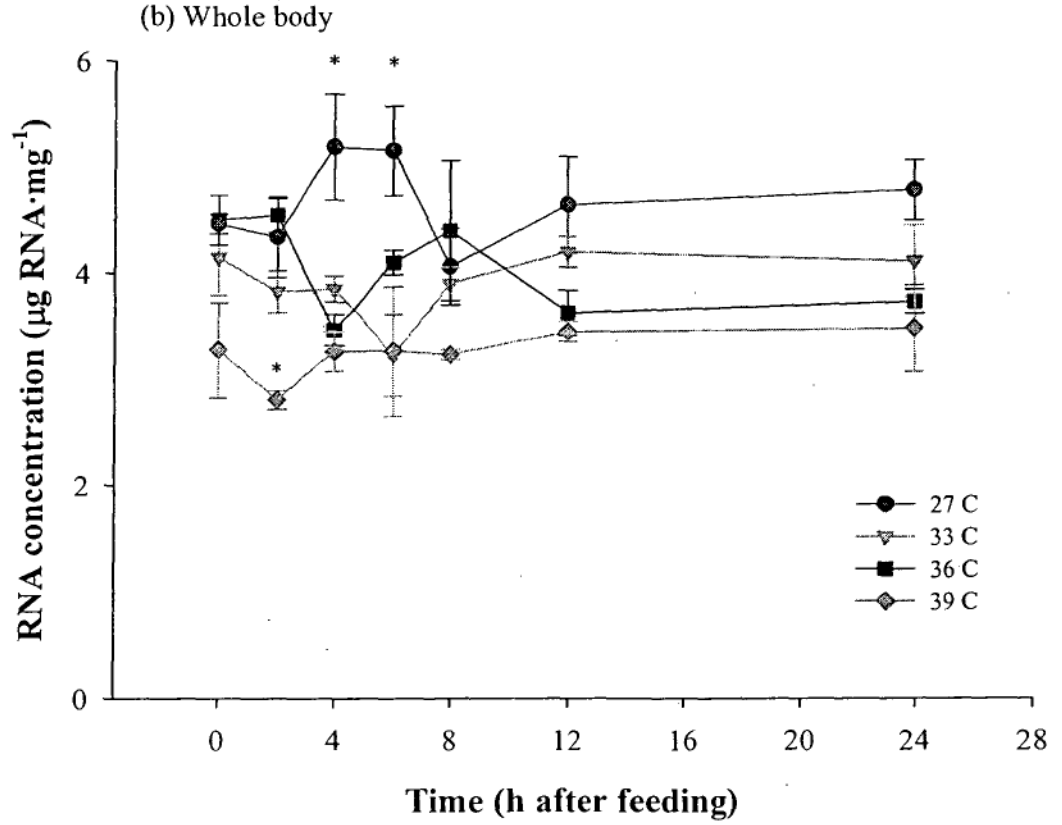
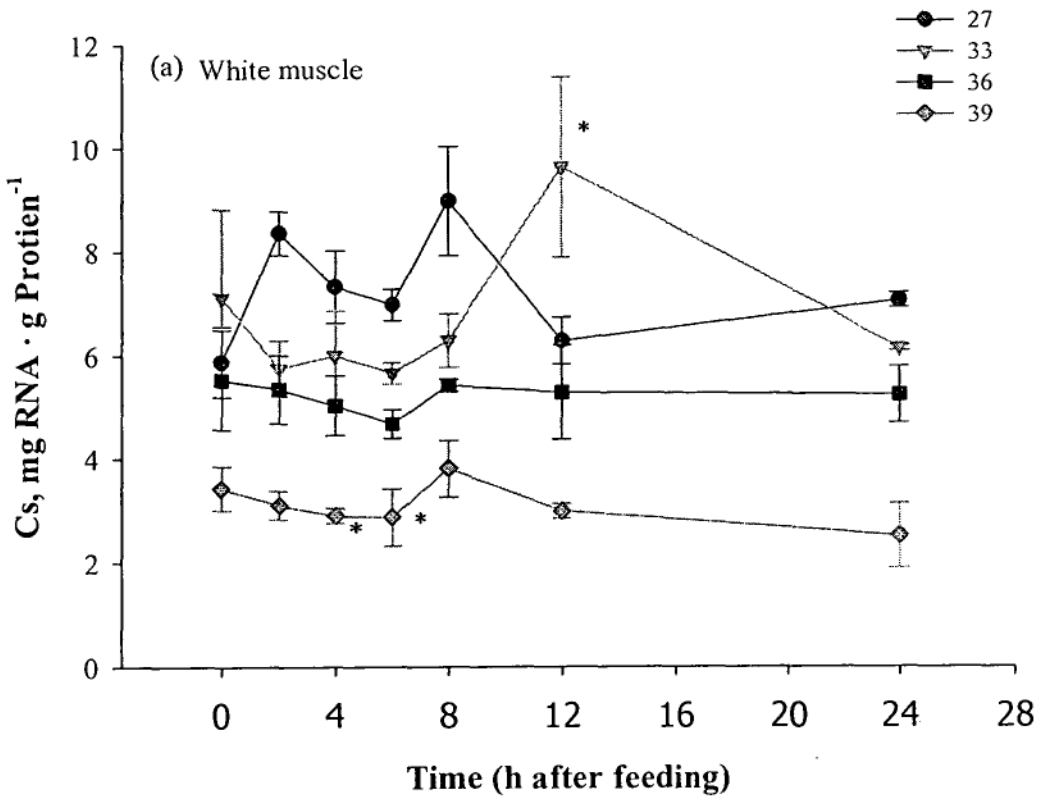
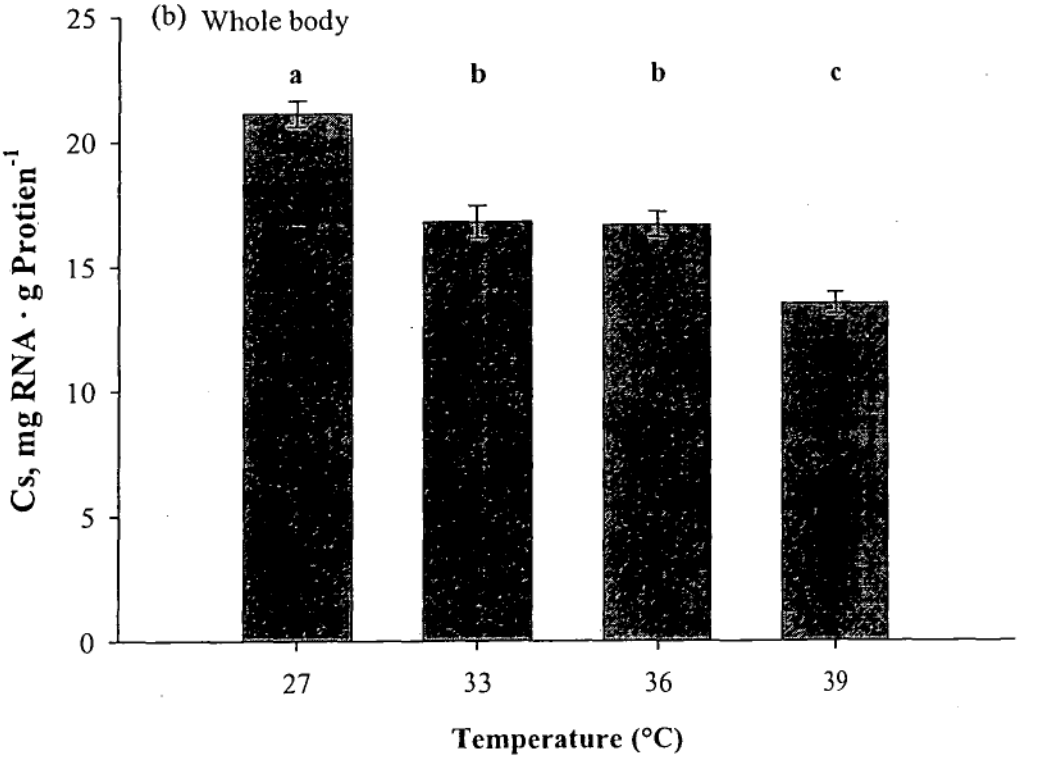

(b) Whole body
RNA concentration (µg RNA·mg-1)
6
4
2
0
*
*
*
27 C
33 C
36 C
39 C
0
4
8
12
16
20
24
28
Time (h after feeding)

Figure 6.6. The effect of feed intake on Cs (mg RNA • g Protein $^{-1}$) for (a) white muscle for juvenile barramundi, *Lates calcarifer*, over 24 h after a final meal at 27, 33, 36 and 39°C. An asterisk (*) indicate significant 2-way interaction is between the temperature and the time after feeding ($p<0.05$). (b) The effect of temperature on the Cs (mg RNA • g Protein $^{-1}$) for whole body. Letters indicate a significant difference between temperatures ($p<0.05$).


(a) White muscle
27
33
36
39
Cs, mg RNA · g Protien-1
Time (h after feeding)
0
2
4
6
8
10
12
0
4
8
12
16
20
24
28
*
*
*


(b) Whole body
Cs, mg RNA · g Protien-1
Temperature (°C)
a
b
b
c
0
5
10
15
20
25
27
33
36
39

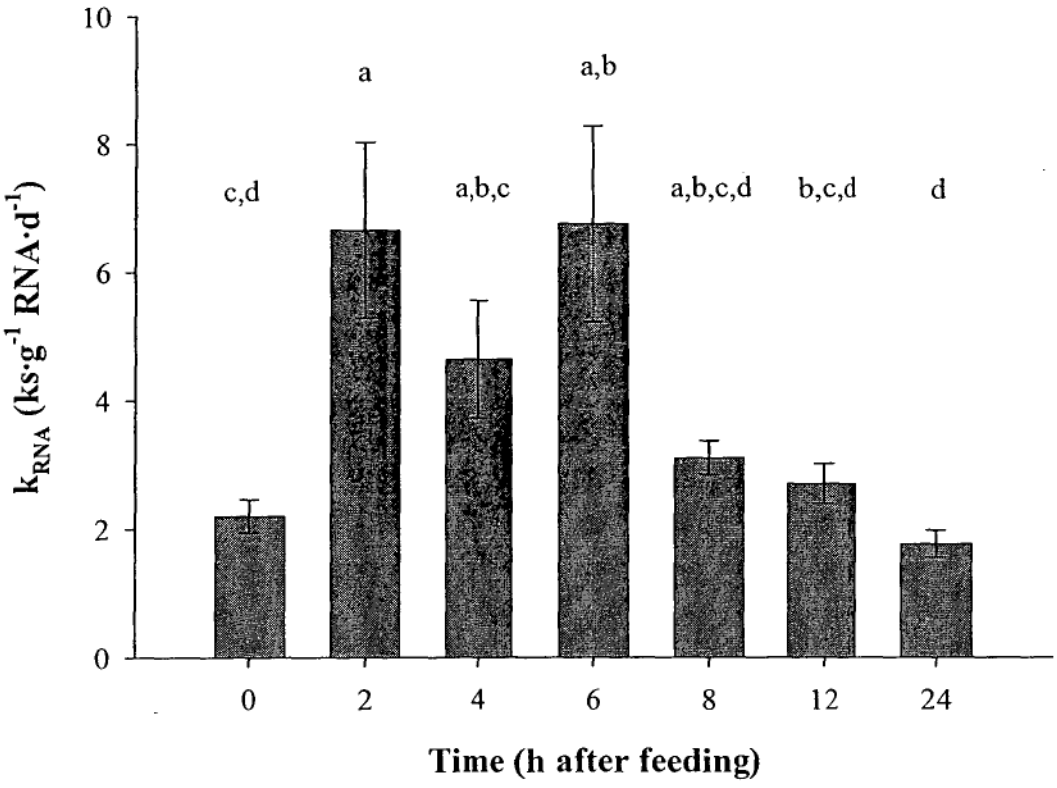
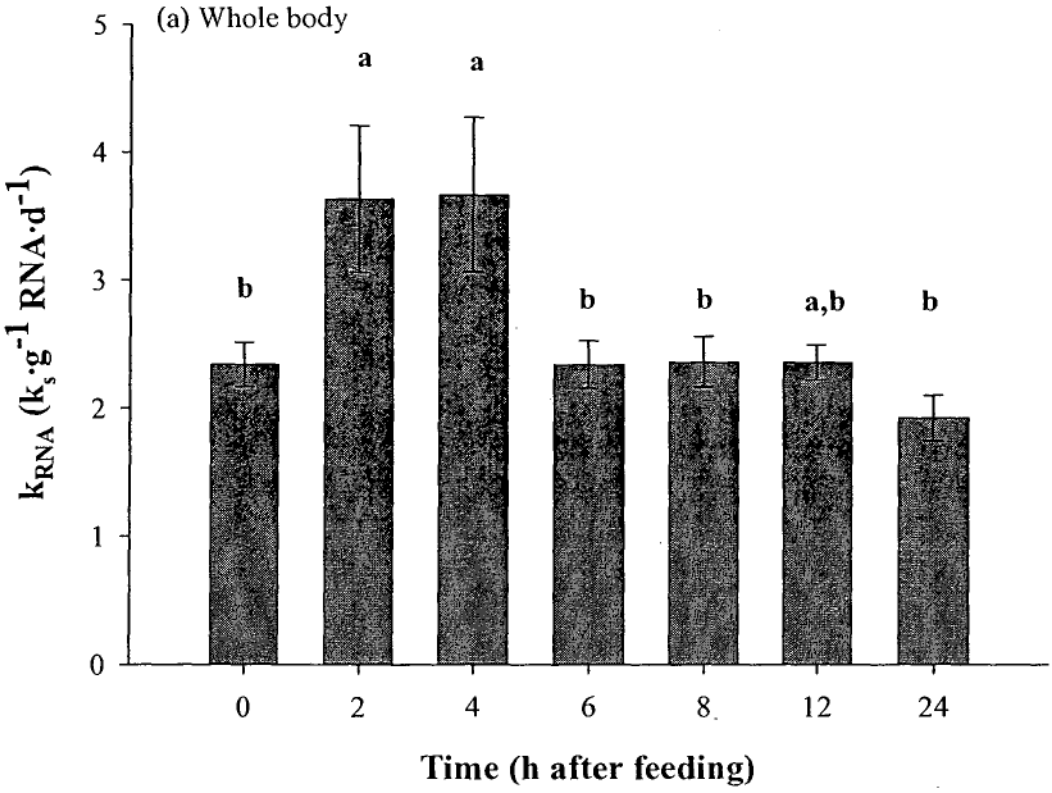


Figure 6.7. The effect of feed intake on ribosomal activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$) in the white muscle for juvenile barramundi, *Lates calcarifer*. Letters indicate a significant difference between time after feeding ($p<0.05$).

Figure 6.8. The effect of (a) feed intake and (b) temperature on the ribosomal activity (k_{RNA}, $k_s \cdot g^{-1}$ RNA $\cdot d^{-1}$) in the whole body for juvenile barramundi, *Lates calcarifer*. Letters indicate a significant difference between time after feeding or temperature ($p<0.05$).


(a) Whole body
k_{RNA} ($k_s \cdot g^{-1}$ RNA$\cdot d^{-1}$)
0
1
2
3
4
5
b
a
a
b
b
a,b
b
0
2
4
6
8
12
24
Time (h after feeding)

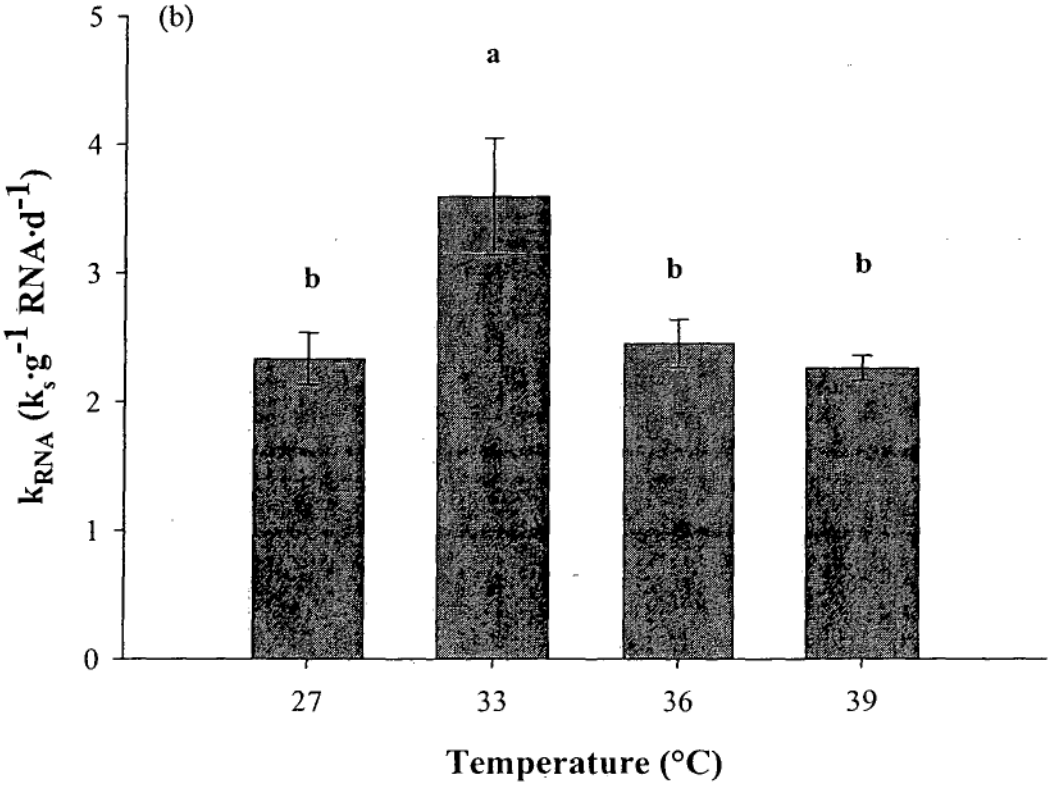

(b)
k_{RNA} ($k_s \cdot g^{-1}$ RNA$\cdot d^{-1}$)
0
1
2
3
4
5
b
a
b
b
27
33
36
39
Temperature (°C)

Figure 6.9.. The relationship between protein intake (k_c, $\%\cdot d^{-1}$) and (a) protein growth rate (k_g, $\%\cdot d^{-1}$). Protein intake and protein growth were determined from the preceding growth trial (Chapter 3), (b) Predicted protein growth (Pre-k_g, $\%\cdot d^{-1}$), protein growth predicted from the protein intake on the day of the flooding dose and (c) protein synthesis (k_s, $\%\cdot d^{-1}$) measured that day. The protein intake was based on feed intake on the day of the flooding dose experiment in figures 6.9b and c.

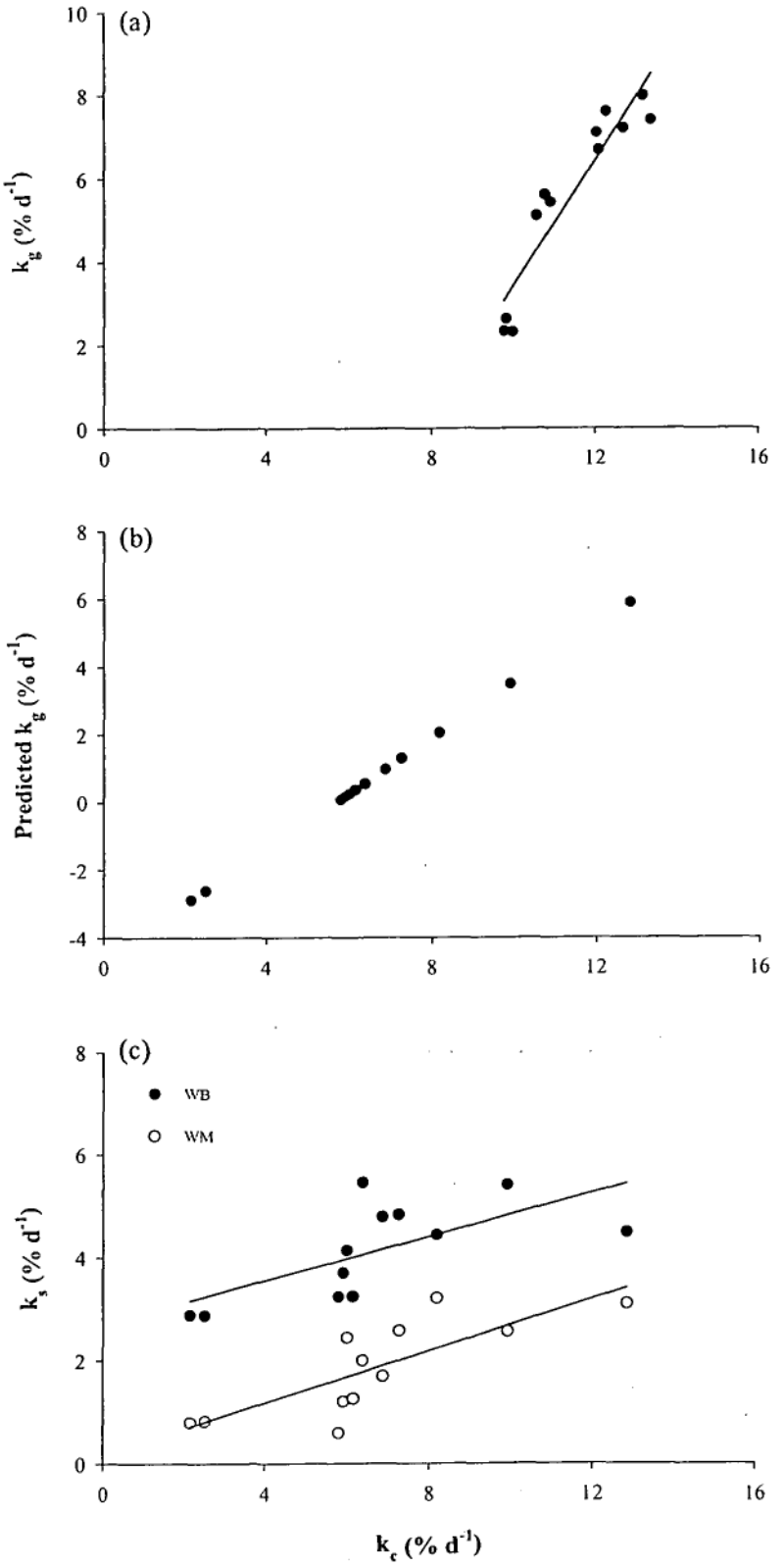
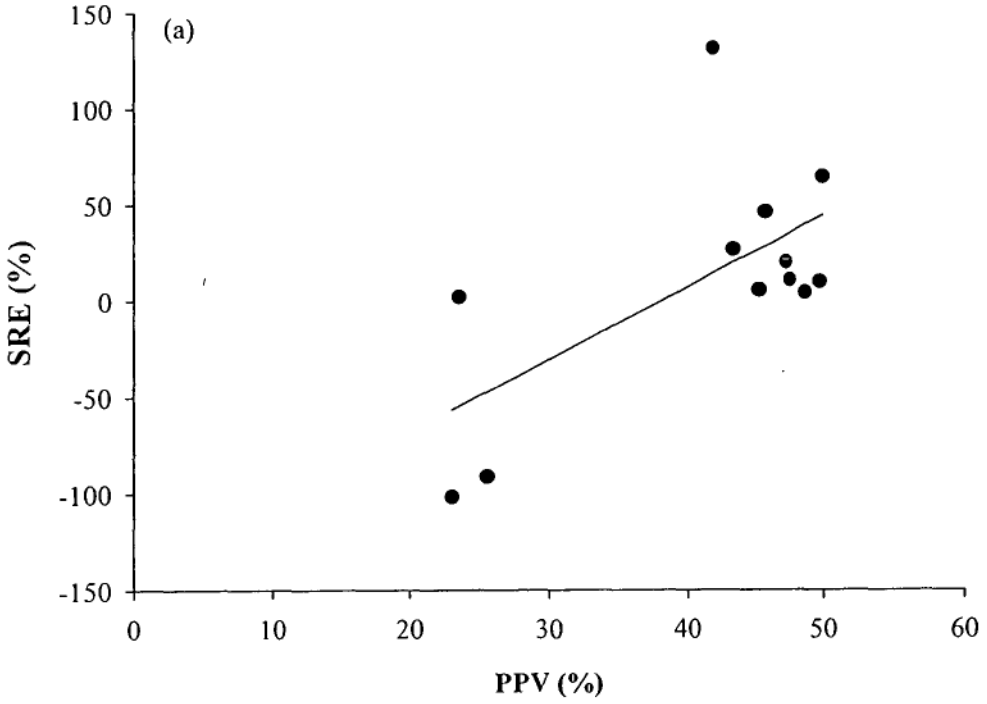
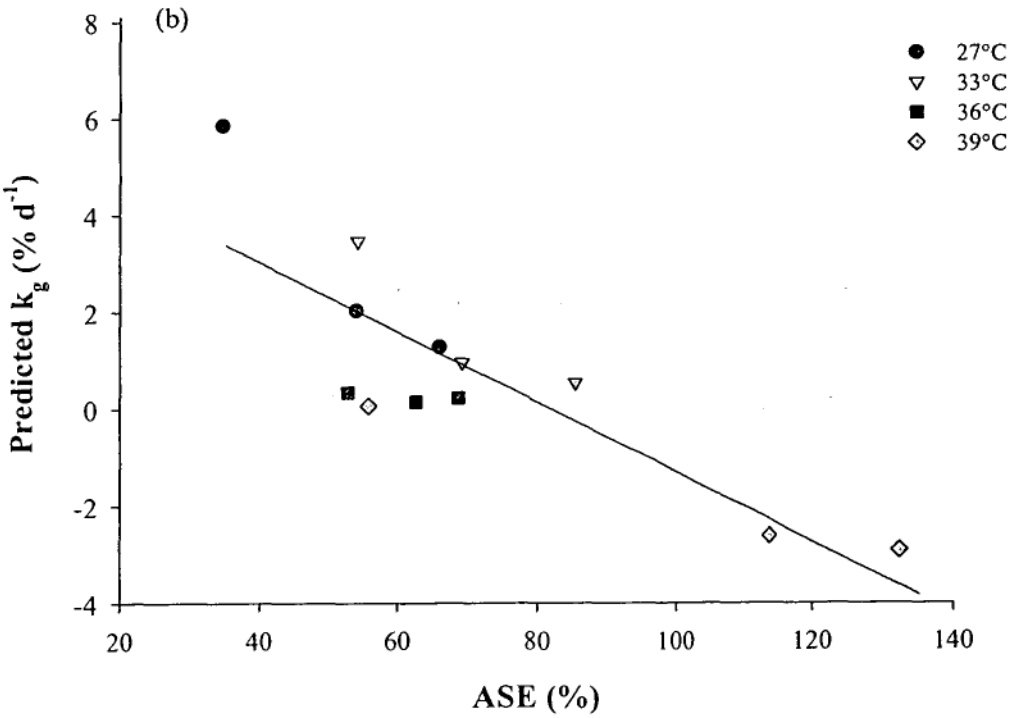

(a)
k_g (% d^{-1})
(b)
Predicted k_g (% d^{-1})
(c)
WB
WM
k_s (% d^{-1})
k_c (% d^{-1})

Figure 6.10. The relationship between (a) productive protein value (PPV, %) and synthesis retention efficiency (SRE, %) for juvenile barramundi, *Lates calcarifer* across all temperatures tested. The relationship was described by the equation, SRE = 3.76PPV - 143.41 (r^2=0.40; p=0.03; n=12). PPV data was obtained from Chapter 3. The relationship between (b) anabolic stimulation efficiency (ASE, %) and the predicted growth (Pre-k_g, %•d^{-1}) was described by the equation Pre-k_g = -0.07ASE + 5.90 (r^2= 0.71; p<0.001; n=12). Pre-k_g was calculated from the protein intake on the day of the flooding dose experiment.


(a)
SRE (%)
PPV (%)
150
100
50
0
-50
-100
-150
0
10
20
30
40
50
60


(b)
Predicted k_g (% d^{-1})
ASE (%)
27°C
33°C
36°C
39°C
8
6
4
2
0
-2
-4
20
40
60
80
100
120
140

6.4. DISCUSSION

This study is the first to examine protein synthesis in a number of tissues and the whole body for juvenile barramundi at temperatures which encompassed and exceeded the optimal range for growth efficiency. As yet, no other study has examined *in vitro* protein synthesis at extreme high temperatures. These results show that feed intake is the driving force of protein synthesis through anabolic stimulation efficiency however at high temperatures protein synthesis was not translated into growth due to high protein turnover.

6.4.2. Validations of flooding dose method

Two assumptions of the flooding dose method are, that a linear increase exists in the protein bound specific radioactivity of the ^{3}H-phenylalanine over the incubation time and that the free pool specific radioactivity of the ^{3}H-phenylalanine remains constant over this time period (Houlihan, *et al.*, 1988). In the present study, these assumptions were confirmed in the white muscle (Fig 6.1a and b) but were not upheld for the liver, stomach or digestive tract. These three tissues have high rates of protein synthesis (Houlihan, *et al.*, 1988; McMillan and Houlihan, 1988; Lyndon, et al., 1992) which are sensitive to feed intake (reviewed by Carter and Houlihan, 2001), contribute a large proportion to the whole body protein synthesis and the SDA from the collective post-prandial events from these tissues (Jobling, 1981). It is apparent from the validation figures (See Appendix I), that the incubation time was to long for these tissues, resulting in a non-significant relationship for the protein bound liver specific radioactivity (AI, Fig. 1a) and significantly decreasing free pools over the incubation period for the stomach (AI, Fig. 2b) and digestive tract (AI, Fig. 3). The different incubation times required for accurate measurement of protein synthesis in different tissues has been noted (Foster, *et al.*, 1992), however when trying to measure a number of different tissues in one fish an average time must be chosen. This is further complicated when examining different temperatures, as temperatures

increases (within the thermal tolerance range) the rate of synthesis increases, increasing the rate which the free pool is depleted.

6.4.1. Protein synthesis

It has been well established that feed intake will elicit a significant response in protein synthesis (reviewed by Houlihan, *et al.*, 1995a). In previous experiments on barramundi (Chapter 5), feed intake was significantly higher at 33°C ($p = 0.048$, $n=3$) by ~40%, the feed intake at 27°C was not significantly higher ($p = 0.097$, $n=3$) however the mean feed intake in the present study was only 60% of the previous experiment. These differences in feed intake were reflected in the white muscle and whole body protein synthesis between the two experiments. Feed intake stimulates protein synthesis through anabolic stimulation efficiency (ASE), therefore the higher the feed intake the greater the ASE. More specifically, Millward (1989) defined this as the anabolic drive, a regulatory stimulus of metabolism without which, protein utilization would not occur, furthermore this response only occurs under conditions where maintenance requirements are met. In the present study, a significant relationship between the feed intake and the WM and WBk_s existed and the slope of the lines represents the ASE for the tissue (Houlihan, *et al.*, 1993). As yet, no other studies have compared these regressions for the WM and WB tissues in fish. There was no difference between these slopes indicating a common response between the white muscle and whole body to feed intake.

The increase in protein synthesis after feeding occurs rapidly and in the present study, at 2 and 4 h after feeding, an increase in whole body protein synthesis was evident at 27 and 33°C. This increase was expected and has previously been observed in barramundi (Chapter 5) amongst other species (Fauconneau, *et al.*, 1989; McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992). The duration of the whole body post-prandial event was shorter than previously determined for barramundi and in the present study the protein synthesis returned to pre-feeding levels at 6 h after feeding.

The magnitude of SDA is dependant upon the size of the ration (Jobling, 1981; Katersky, *et al.*, 2006) and feeding regime (McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992) with greater feed intake being more energetically demanding resulting in a larger SDA event. Fauconneau *et al.* (1989) found that continually fed Atlantic salmon did not exhibit such intense increases in protein synthesis after feeding as starved fish. The difference in the post-prandial effect in this study as compared with common temperatures in previous experiments (Chapter 5) is attributed to the differences in the feed intake.

In the present study, the WM and WBk_s were not significantly different for temperatures 27, 33 and 36°C but significantly declined at 39°C, a temperature which exceeds the optimal temperature range for growth efficiency (25-37°C, Chapter 4). These results correspond with a plateau observed in feed intake and growth efficiency at temperatures of 27, 33 and 36°C as well as with the low feed intake and significantly lower growth rate of fish held at 39°C (Katersky and Carter, 2005). The relationship between feed intake and protein synthesis becomes more complicated when temperature effects are considered. Foster *et al.* (1992) isolated the effects of temperature on the protein synthesis of cod by feeding similar rations to two different temperatures. He found that similar rates of protein synthesis occur betwèen temperature and that protein synthesis rates were dependant upon an increase in RNA concentration to compensate for the low ribosomal activity which occurrs at low temperatures. A different scenario usually occurs when fish are fed *ad libitum* at different temperatures (as was attempted in this study), differences in feed intake occur (Jobling, 1997; Katersky and Carter, 2005) which are then translated to increased protein synthesis at increasing temperatures (Watt, *et al.*, 1988; Mathers, *et al.*, 1993; McCarthy, *et al.*, 1999). This increase in protein synthesis with temperature is observed at temperatures with the optimal thermal tolerance range.

6.4.3 Protein turnover

The relationship between growth efficiency and synthesis retention efficiency indicate that with higher growth efficiency there is a higher retention of synthesized proteins (Houlihan, *et al.*, 1989; Carter, *et al.*, 1993, this study). This relationship is dependant upon the availability and the balance of dietary nutrients (Carter and Houlihan, 2001). When the protein:energy in the diet is not balanced, greater catabolism of amino acids occur and the efficiency of growth is reduced due to an increase in energy expenditure (Carter and Houlihan, 2001). As stated above, feed intake stimulates protein synthesis through anabolic stimulation efficiency (Millward, 1989; Houlihan, *et al.*, 1993). When ASE is correlated with k_g, there was a significant negative linear relationship. This was related to the acclimation temperature, even though there was no significant difference between temperatures. When the ASE data is examined high variability exists among the temperature replicates especially at 39°C and this was attributed to the higher feed intake in one of the replicates. In Fig. 13b, temperatures were identified and it is clear that the ASE at 39°C is driving the regression. This relationship also shows that at 39°C, where high ASE was occurring there was low growth indicating that high protein turnover was taking place. It can be substantiated by increased feed intake not only stimulate stimulates protein synthesis but also protein degradation (reviewed by Millward, 1989).

6.4.4. Mechanism of Protein Synthesis

It is clear from the present study that protein synthesis is driven through increases in ribosomal activity (k_{RNA}) stimulated by feeding and not by the concentration of RNA or Cs. These findings are in agreement with previous work on barramundi (Chapter 5), cod (Lyndon, *et al.*, 1992) and rainbow trout (McMillan and Houlihan, 1989) where from the time of feeding the k_{RNA} followed similar patterns for each the WM and WB protein synthesis and the Cs remained relatively stable. Thus, indicating that

feeding stimulates RNA translation, the mechanism of protein synthesis (Taylor and Brameld, 1999), enabling synthesis to occur at a higher rate while the ratio of RNA to protein remains constant. The RNA to protein ratio has been termed the capacity for protein synthesis because the relative level of Cs is directly related to protein synthesis and therefore growth potential (McMillan and Houlihan, 1989; Lyndon, *et al.*, 1992). In the present study, the WM and WB Cs were very similar to those previously determined for barramundi (Chapter 5). Suggesting that protein synthesis in the present study had the capacity to attain similar levels as in Chapter 5. This further identifies feed intake as the limiting factor. The temperature effect on Cs were evident at 39°C, where a significantly lower ratio was present, implying that at extreme high temperatures (nearing the upper thermal tolerance limit) that there was a lower capacity for synthesizing proteins (and therefore growth) reflected in significantly lower k_s. Although no studies to date have examined protein synthesis at temperatures near the upper thermal tolerance limit, the limited capacity at high temperatures is logical in relation to the thermally stressed environment where low oxygen availability, low energy intake and an exponential increasing metabolic rate would be present (Jobling, 1994).

6.5. REFERENCES

Ashford, A.J., Pain, V.M., 1985. Effects of diabetes in the rates of synthesis and degradation of ribosomes in rat muscle and liver *in vivo*. Journal of Biological Chemistry 261, 4059-4065.

Barlow, C.G., Pearce, M.G., Rodgers, L.J. and Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). Aquaculture 138, 159-168.

Carter, C.G., Houlihan, D.F., 2001. Protein synthesis. In: Wright, P., Anderson, P. (Eds.), Fish Physiology, Vol. 20: Nitrogen Excretion. Academic Press, New York, NY, pp. 31-75.

Carter, C.G., Houlihan, D.F., Brechin, J., McCarthy, I.D., 1993. The relationship between protein intake and protein accretion, synthesis and retention efficiency for

individual grass carp, *Ctenopharyngodon idella* (Valenciennes). Canadian Journal of Zoology 71, 392-400.

Carter, C.G., Houlihan, D.F., Kiessling, A., Medale, F., Jobling, M., 2001. Physiological effects of feeding. In: Houlihan, D.F., Boujard, T., Jobling, M. (Eds.), Food Intake in Fishes. Blackwell Scientific, Oxford, pp. 297-331.

Fauconneau, B., Arnal, M., 1985. In vivo protein synthesis in different tissues and the whole body of rainbow trout (*Salmo gairdnerii* R.). Influence of environmental temperature. Comparative Biochemistry and Physiology Part A 82, 179-187.

Fauconneau, B., Breque, J., Bielle, C., 1989. Influence of feeding on protein metabolism in Atlantic salmon (*Salmo salar*). Aquaculture 79, 29-36.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod. Canadian Journal of Zoology 70, 2095-2102.

Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissue by injection of [^{3}H] phenylalanine. Biochemical Journal 192, 719-723.

Houlihan, D.F., 1991. Protein turnover in ectotherms and its relationship to energetics. In: Gilled, R. (Ed.), Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin Heidelberg, pp. 1-43.

Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effects of body size. Physiological Zoology 59, 482-493.

Houlihan, D.F., Hall, S.J., Gray, C., 1989. Effects of ration on protein turnover in cod. Aquaculture 79, 103-110.

Houlihan, D.F., Mathers, E.M., Foster, A., 1993. Biochemical correlates of growth rate in fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 45-68.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995a. Protein synthesis in fish. In: Hochachka, P., Mommsen, P. (Eds.), Biochemistry and Molecular Biology of Fishes, pp. 191-220.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995b. Protein turnover in animals. In: Walsh, P.J., Wright, P. (Eds.), Nitrogen metabolism and excretion. CRC Press, New York, pp. 1-32.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45, 951-964.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. Journal of Fish Biology 18, 385-400.

Jobling, M., 1983. Towards an explanation of specific dynamic action (SDA). Journal of Fish Biology 23, 529-555.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Katersky, R.S., Carter, C.G., 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Katersky, R.S., Peck, M.A., Bengtson, D.A., 2006. Oxygen consumption of newly settled summer flounder, *Paralichthys dentatus* (Linnaeus, 1766). Aquaculture 257, 249-256.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Lyndon, A.R., Houlihan, D.F., Hall, S.J., 1992. The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. Journal of Comparative Physiology B 162, 209-215.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., Burren, L.J., 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. Journal of Fish Biology 43, 245-263.

McCarthy, I., Houlihan, D.F., 1997. The effect of temperature on protein metabolism in fish: the possible consequences for wild Atlantic salmon (*Salmo salar* L.) stocks in Europe as a result of global warming. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 51-77.

McCarthy, I.D., Moksness, E., Pavlov, D.A., Houlihan, D.F., 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Sciences 56, 231-241.

McCue, M.D., 2006. Specific dynamic action: A century of investigation. Comparative Biochemistry and Physiology - Part A 144, 381-394.

McMillan, D.N., Houlihan, D.F., 1989. Short-term responses of protein synthesis to re-feeding in rainbow trout. Aquaculture 79, 37-46.

Millward, D.J., 1989. The nutritional regulation of muscle growth and protein turnover. Aquaculture 79, 1-28.

Sugden, P.H., Fuller, S.J., 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochemical Journal 273, 21-37.

Taylor, P.M., Brameld, J.M., 1999. Mechanisms and regulation of transcription and translation. In: Lobley, G.E., White, A., MacRae, J.C. (Eds.), Protein metabolism and nutrition. Wageningen Pers, Wageningen, pp. 25-50.

Watt, P.W., Marshall, P.A., Heap, S.P., Loughna, P.T., Goldspink, G., 1988. Protein synthesis in tissues of fed and starved carp, acclimated to different temperatures. Fish Physiology and Biochemistry 4, 165- 173.

CHAPTER 7

GENERAL DISCUSSION

7.1. OVERVIEW OF THESIS

This thesis has examined the effects of feed intake, growth efficiency and protein metabolism across the thermal tolerance range for barramundi. Numerous factors (both exogenous and endogenous) will affect the growth efficiency of fish, from dietary nutrition to the environment, fish size to the sex of the fish. Of all of these parameters, temperature is the most important exogenous factor influencing metabolic efficiency. The major findings of each research chapter were as follows:

Chapter 2: Protein synthesis in the whole body was 4x greater than the white muscle ks. Regardless of temperature, at 24 h after feeding the fractional rate of protein synthesis did not differ over a temperature range of 21 to 33°C.

Chapter 3: Feed intake, growth and growth efficiency for protein and energy plateaued between the temperatures of 27-36°C. It was not until 39°C that a significant decline was observed.

Chapter 4: The optimal temperature of 31°C was determined from models of feed intake, growth and growth efficiency. Interestingly, the optimal temperature ranges were ~10 times wider for growth efficiency than for feed intake and growth.

Chapter 5: Protein synthesis was driven by ribosomal activity in all tissues. In all tissues peak k_s was 4 h after feeding. No differences in protein synthesis occurred between the temperatures of 27 and 33°C, which correspond to a plateau in growth. At low temperature (21°C), feed intake did not stimulate post-prandial protein synthesis.

Chapter 6: Protein synthesis was directly influenced by feed intake. Ribosomal activity was the driving force of protein synthesis. At extreme high temperatures no post-prandial event in protein synthesis was demonstrated, partly due to low feed intake.

The purpose of this final chapter is to model the protein metabolism data generated in chapters 5 and 6, along with the protein consumption and growth data from Chapters 2 and 3 into a series of regressions, in order to better understand the relationships between measures of protein turnover. Models were also generated to predict protein accretion (k_g) from protein consumption (k_c) on the days of the flooding dose injection in order to determine the effect of temperature on the protein turnover over the entire thermal tolerance range for barramundi.

7.2. PROTEIN TURNOVER MODELS

A number of relationships have been described by Houlihan *et al.*, (1993; 1995) to highlight the impact of protein intake on the growth and protein synthesis in fish as well as on RNA concentration (expressed as $\mu g\ RNA \cdot mg^{-1}$ and as $mg\ RNA \cdot protein^{-1}$) and its impact on k_s. Originally, it was thought that these relationships held true only for a specific thermal regime (Houlihan, *et al.*, 1993). The data from this thesis suggests that these relationships hold true irrespective of temperature.

The effect of feed intake on protein synthesis is an area of research which has received a great deal of attention (Houlihan, *et al.*, 1988; Millward, 1989; Carter, *et al.*, 1993; Mathers, *et al.*, 1993; Meyer-Burgdorff and Rosenow, 1995; McCarthy, *et al.*, 1999). One of the main findings of this thesis was that feed intake drives protein synthesis. This was clearly demonstrated by the combined data (Fig. 7.1a) for which protein intake explained 75% of the variation in protein synthesis across a range of temperatures (21-39°C). The data allowed comparison between white muscle and whole body (Fig. 7.1b) interestingly the lines appeared to diverge at high

consumption rates indicating that white muscle has a lower capacity to respond to feed intake than the whole fish. However, when tested by ANCOVA the lines were not significantly different ($F = 0.35$, $df = 1, 17$ $p = 0.56$) and the relative stimulation of protein synthesis is the same as protein intake increases. It should be pointed out that these data were collected from barramundi across the thermal tolerance range (21-39°C). As noted earlier these relationships were thought to hold true for a specific thermal regime (Houlihan, *et al.*, 1993), however these data show that regardless of environmental temperature increased protein intake increased protein synthesis. Over 75% of the variation seen in k_s was explained by the variation in k_c. Temperature obviously plays a significant role in the amount of feed consumed and when fish are fed *ad libitum* to satiation differences are clearly seen in rates of synthesis (Chapter 5).

The relationship between consumption (k_c) and growth (k_g) has generally been expressed as a linear regression (Carter, *et al.*, 1993; Lupatsch, 2001). The data presented in this chapter were from the protein intake collected from growth experiments described in Chapters 2 and 3. It is understood that this relationship may not be truly linear, although only one point at the highest ration appears to diverge away from being on a linear relationship (Fig. 7.2a), however a linear regression was used here to analyse the data. Growth rates can be predicted from the feed intake on the day the k_s measurements were made in order to relate the protein synthesis measurement to a predicted growth rate (Fig. 7.2b). This was done to account for low feed intake in Chapter 6 on the day of the flooding dose (significantly lower than the immediately preceding growth trial). There was a significant relationship between k_s as measured and predicted growth rates for replicate tanks ($F = 56.99$; $df = 1,19$; $p<0.001$, Fig. 7.2b) and across the thermal tolerance range ($F = 27.22$; $df = 1,6$; $p = 0.003$, Fig.7.2c). When mean rates (± s.e.) of k_s and k_g are examined across the thermal tolerance range, the linear regression indicates the synthesis retention efficiency, which increased with increased protein synthesis (Fig. 7.2c).

From the predicted measures of growth, an estimate of protein turnover can be determined at each temperature. Real data on the protein synthesis is combined with the predicted k_g (based on the real k_c) to determine the predicted k_d from Chapters 5 and 6 (Fig 7.3 a and b, respectively). This is a representation of what might have occurred had the fish consumed similar rations on the day of the protein synthesis measurements as they had throughout the growth trials. It does however, allow some interesting issues to be explored. At the extreme low temperature (21°C) feed intake was not high enough to meet the maintenance requirement and weight loss would be occurring. At this temperature, the physiological processes are functioning at a depressed rate, no post-prandial effect will occur (Chapter 5) meaning the feed intake has little influence on the metabolic rate and protein turnover is equal to the protein synthesis (protein turnover as defined by Houlihan, *et al.*, 1993). As the temperature increases to 27°C in both experiments k_g is increased and protein accretion occurs, indicating that protein turnover is measured by protein degradation. In both figure 7.3a and 7.3b, at 33°C estimates of k_d are higher than that of k_g, indicating that growth is occurring but that the rate of turnover is high with lower retention of the synthesized proteins than at 27°C. In this scenario the protein turnover is dependant upon the protein degradation. At 36°C (Fig 3b) k_s and k_d are nearly equal and growth is minimal, synthesis retention is low and turnover high. Under these conditions, the fish are just above the steady state of maintenance where $k_s = k_t = k_d$ (Houlihan, *et al.*, 1993). At 39°C, near the upper thermal tolerance limit weight loss is occurring as it did at 21°C. Again growth is limited by food intake and turnover is equal to protein synthesis. Although k_s increases as the temperature increased from 21 to 27°C a plateau is reached at temperature above 27°C, it is at this point where protein degradation becomes the dominant force in turnover and ultimately determines the growth of the fish. Under these predictions the optimal temperature for protein accretion would be 27°C.

The RNA concentration expressed as μg RNA·mg^{-1} and as Cs (mg RNA· protein^{-1}) relate well to protein synthesis (Figure 7.4a and b). Protein synthesis is dependant

upon the translation of ribosomal activity (Taylor and Brameld, 1999) and therefore it was not surprising that significant relationships occurred (RNA concentration, WBk_s = 2.23[RNA] -4.67 (r^2 = 0.67, n=7, p=0.03, Fig. 7.4a) and Cs, WBk_s=0.61Cs -5.78 (r^2 = 0.64, n=7, p=0.03, Fig. 7.4b). Measures of RNA have been utilized as indicators of growth based on the strong relationships between RNA and protein synthesis and protein synthesis and growth (Houlihan, *et al.*, 1993). Both the WB RNA and WB Cs significantly correlate to WB k_s, and have been shown across tissues. However, even though these models do not show a significant relationship for WM there is a trend for a positive relationship between WM RNA concentration and WMk_s (RNA concentration, (r^2 = 0.26, n=7, p=0.24, Fig. 7.4a and Cs, r^2 = 0.52, n=7, p=0.67, Fig. 7.4b). The differences between the white muscle and whole bodies are their relative rates of protein synthesis and concentration of RNA. In previous work, it has been determined that at low temperatures there is an increased concentration of RNA to compensate for low ribosomal activity (Foster, *et al.*, 1992). However, in this thesis this has not been the case for barramundi, there were no differences between the RNA and temperature within each tissue (Chapters 5 and 6). However, it was the RNA concentration which ultimately determined the relative rate of protein synthesis for each tissue irrespective of temperature (Chapters 2, 5 and 6).

The previous models of protein turnover (Fig 7.3a and b) were based on predicted growth data. In figure 7.5, protein synthesis data from Chapter 5 was modelled with 'real' growth data from fish held under the same conditions (i.e. feed intake was similar) in chapter 2 and tell a slightly different story. At all three temperatures 21, 27 and 33°C protein turnover is determined by the protein degradation (Houlihan, *et al.*, 1993), however there is significantly lower growth and synthesis at 21°C, than at 27 and 33°C and these temperatures are not significantly different from one another. This corresponds well with the plateau seen in growth between these temperatures (Katersky and Carter, 2005) . Data from temperatures above 33°C were not able to be included in this model due to low feed intake (Chapter 6) therefore, the temperature protein turnover models are incomplete. However, when the patterns of

protein turnover (Fig. 7.5a) are related to models of PPV (Fig. 7.5b), it can be predicted that protein turnover would follow the same asymmetric response to temperature as other physiological parameters (Jobling, 1994; 1997).

These models demonstrate the influence of feed intake on protein turnover in fish at all temperatures across the thermal tolerance range for barramundi. All of these experiments used a single diet based on the known requirements for juvenile barramundi. Recent research has focused on the effects of lipid on growth at low temperatures (Williams, *et al.*, 2003). It would be interesting to expand on this an determine the optimal protein:energy ratios at each temperature in order to maximizing growth efficiency for protein and energy as well as maximizing the synthesis retention efficiency. Another consideration would be to repeat the experiment in Chapter 5 and attempting to maximize feed intake on the day of the flooding dose in order to measure protein synthesis at complete the models for protein turnover across the thermal tolerance range for barramundi. Nevertheless, this thesis has presented a detained understanding of the protein turnover in an ectotherm over its thermal tolerance range and incorporated an understanding of post-prandial metabolism in a tropical species for the first time.

Figure 7.1. (a) The relationship between k_c (% · d^{-1}) and the daily k_s (%) for replicate tanks was describes by the equation $k_s = 0.635k_c+0.726$ ($r^2 = 0.75$, n= 21, $p<0.001$). (b) The relationship between the mean ± standard error protein intake (k_c, % · d^{-1}) and white muscle (○) and whole body (●) protein synthesis (k_s, % · d^{-1}) for each temperature across the thermal tolerance range for barramundi (21-39°C). The relationship between k_c and WMk_s was described by the equation, $WMk_s = 0.384k_c$-0.101 ($r^2 = 0.78$, n=7, p=0.008) and the relationship between k_c and WBk_s was described by the equation, $WBk_s = 0.668k_c+0.447$ ($r^2 = 0.85$, n=7, p=0.003).

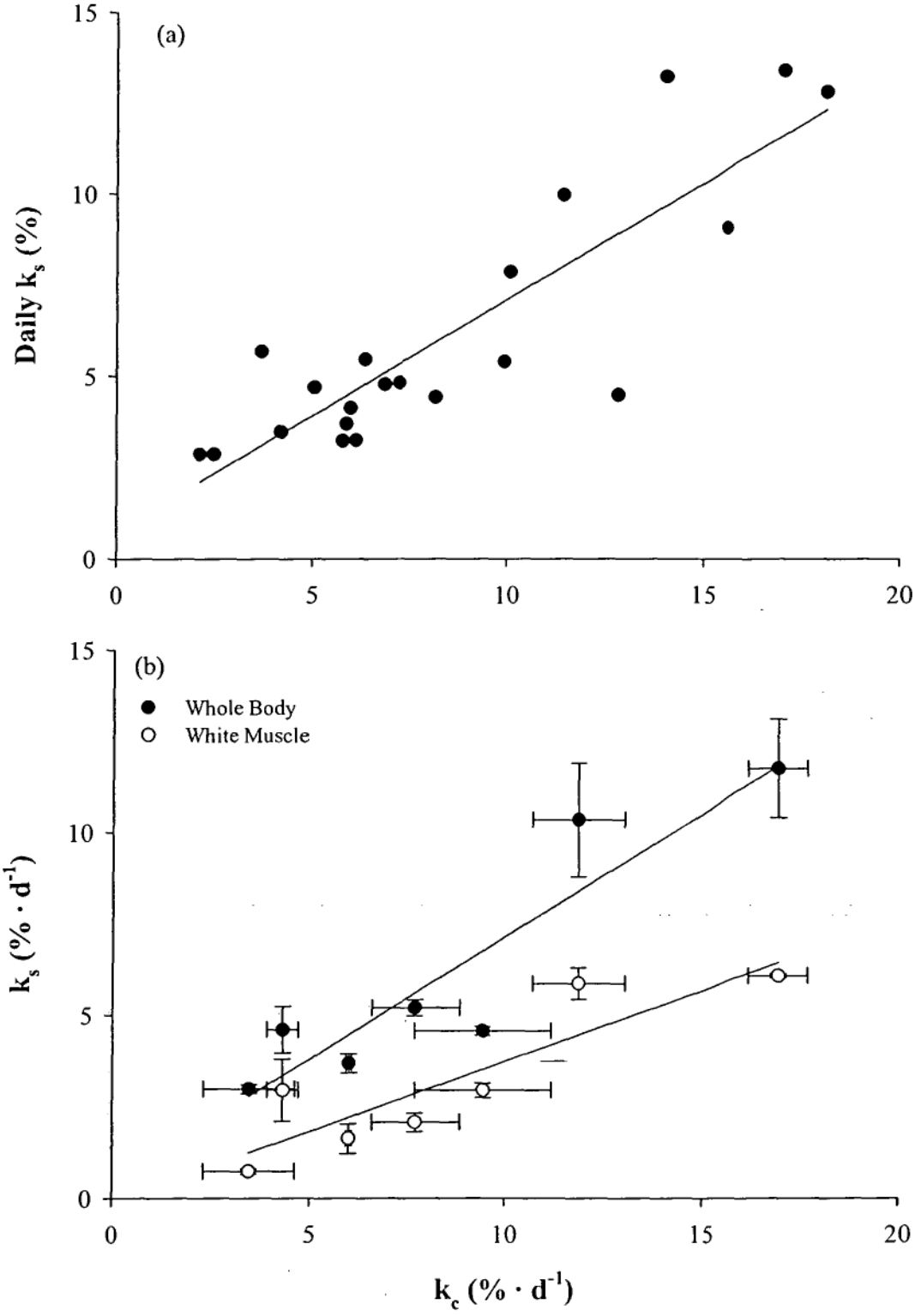

(a)
Daily k_s (%)
15
10
5
0
0
5
10
15
20
(b)
Whole Body
White Muscle
k_s (% · d^{-1})
k_e (% · d^{-1})

Figure 7.2. The relationship between (a) protein intake (k_c, $\%\cdot d^{-1}$) and protein growth rate (k_g, $\%\cdot d^{-1}$) for all data collected in chapters 5 and 6 at temperatures tested across the thermal tolerance range (21-39°C) and was described by the equation $k_g = 0.82k_c - 4.69$ ($r^2 = 0.67$, n= 26, $P<0.001$). (b) The relationship between whole body protein synthesis (WBk_s, $\%\cdot d^{-1}$) and the predicted growth (Pk_g, $\%\cdot d^{-1}$) for each replicate described by the relationship $Pk_g = 0.97WBk_s - 3.64$ ($r^2=0.75$, n=21, $p<0.001$). (c) The relationship between the mean ± standard error WBk_s ($\% \cdot d^{-1}$) and the predicted growth (Pk_g, $\%\cdot d^{-1}$) for each temperature across the thermal tolerance range for barramundi (21-39°C).

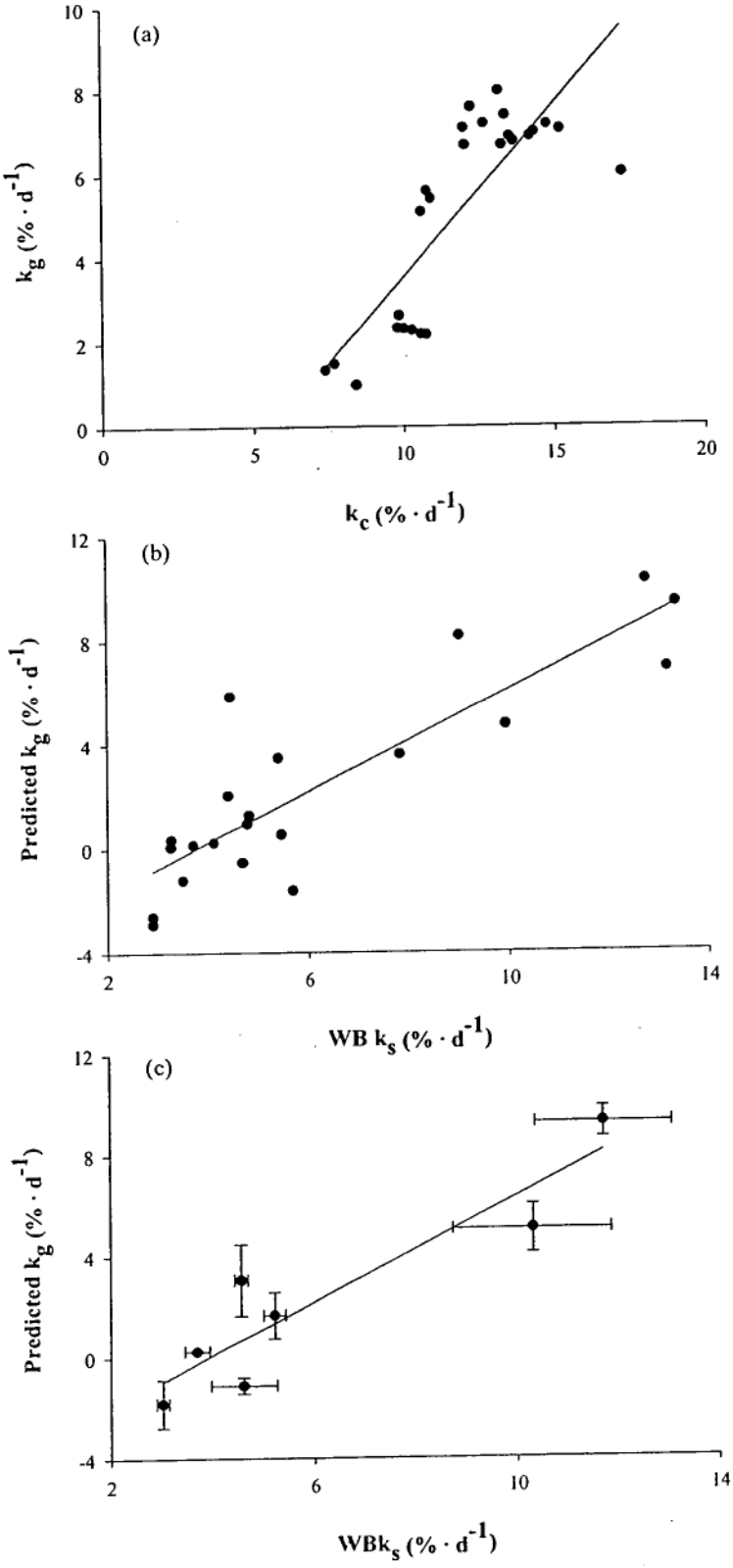

(a)
k_g (% · d^{-1})
k_c (% · d^{-1})
(b)
Predicted k_g (% · d^{-1})
WB k_s (% · d^{-1})
(c)
Predicted k_g (% · d^{-1})
WBk_s (% · d^{-1})

Figure 7.3. The whole body protein synthesis (WBk_s, $\%\cdot d^{-1}$), predicted protein growth (Pk_g, $\%\cdot d^{-1}$) and the predicted protein degradation (Pk_d, $\%\cdot d^{-1}$) for each temperature in (a) Chapter 5 and (b) Chapter 6 in order to estimate protein turnover ($\%\cdot d^{-1}$) at each temperature over the thermal tolerance range (21-39°) for juvenile barramundi.

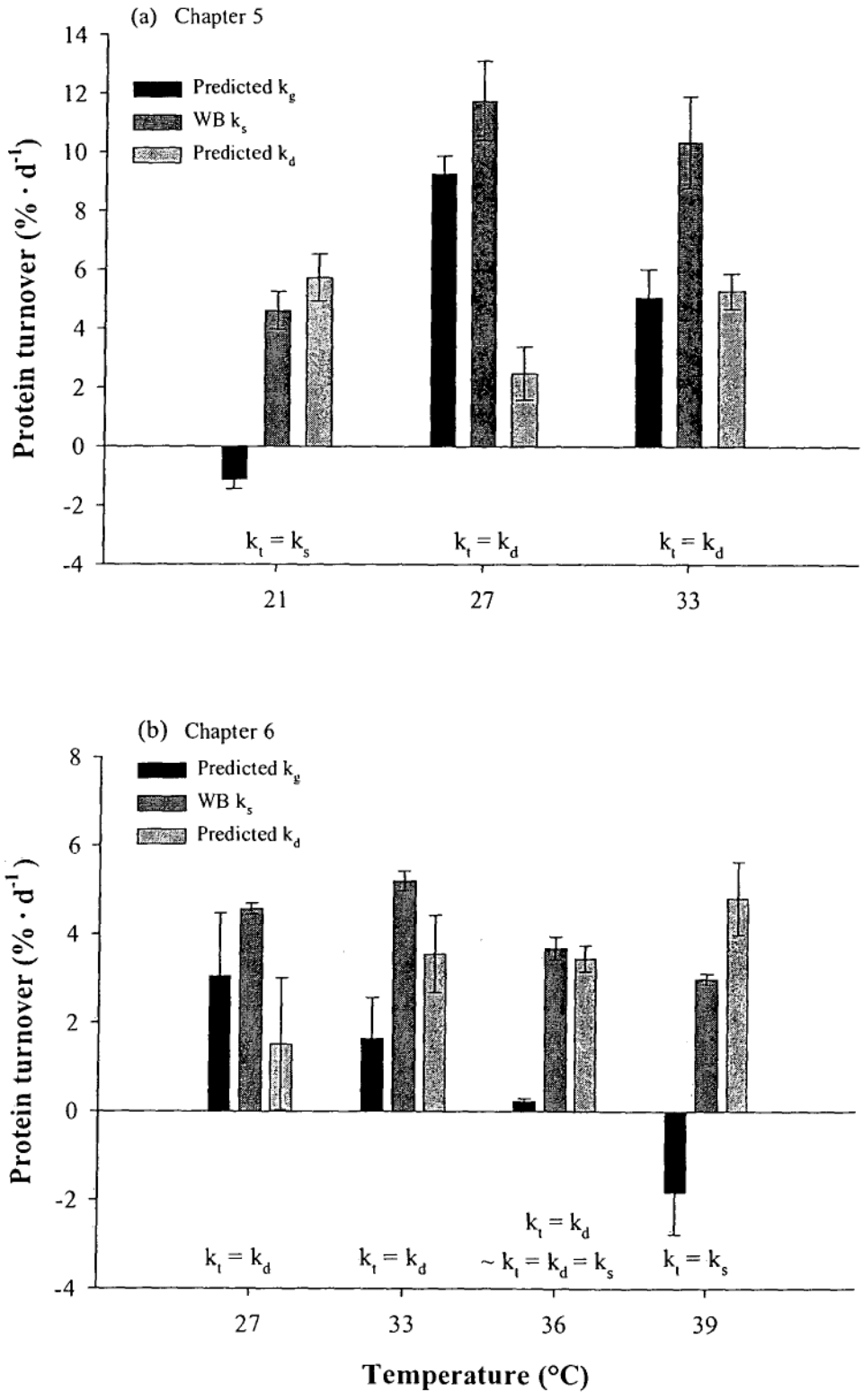
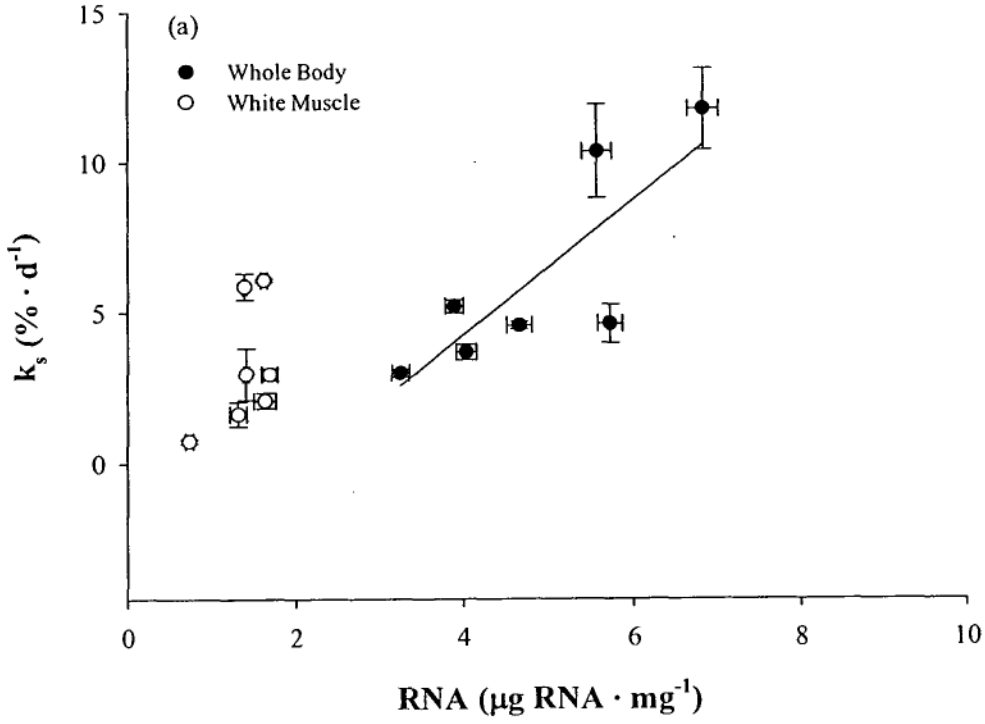

(a) Chapter 5
Predicted k_g
WB k_s
Predicted k_d
Protein turnover (% · d^{-1})
14
12
10
8
6
4
2
0
-2
-4
$k_t = k_s$
$k_t = k_d$
$k_t = k_d$
21
27
33
(b) Chapter 6
Predicted k_g
WB k_s
Predicted k_d
Protein turnover (% · d^{-1})
8
6
4
2
0
-2
-4
$k_t = k_d$
$k_t = k_d$
$k_t = k_d$
~ $k_t = k_s$
$k_t = k_s$
27
33
36
39
Temperature (°C)

Figure 7.4. (a)The relationship between Cs (mg RNA· g Protein^{-1}) and the white muscle (○) and whole body (●) protein synthesis (k_s, %·d^{-1}) for each temperature tested across the thermal tolerance range (21-39°) for juvenile barramundi. A significant relationship occurred for the whole body described by the equation WBk_s=0.61Cs -5.78 (r^2 = 0.64, n=7, p=0.03). (b) The relationship between the concentration of RNA (μg RNA· mg^{-1}) and the white muscle (○) and whole body (●) protein synthesis (k_s, %·d^{-1}) for each temperature tested across the thermal tolerance range (21-39°) for juvenile barramundi. A significant relationship occurred for the whole body described by the equation WBk_s = 2.23[RNA] -4.67 (r^2 = 0.67, n=7, p=0.03).


(a)
Whole Body
White Muscle
15
10
5
0
0
2
4
6
8
10
k_s (% · d^{-1})
RNA (µg RNA · mg^{-1})

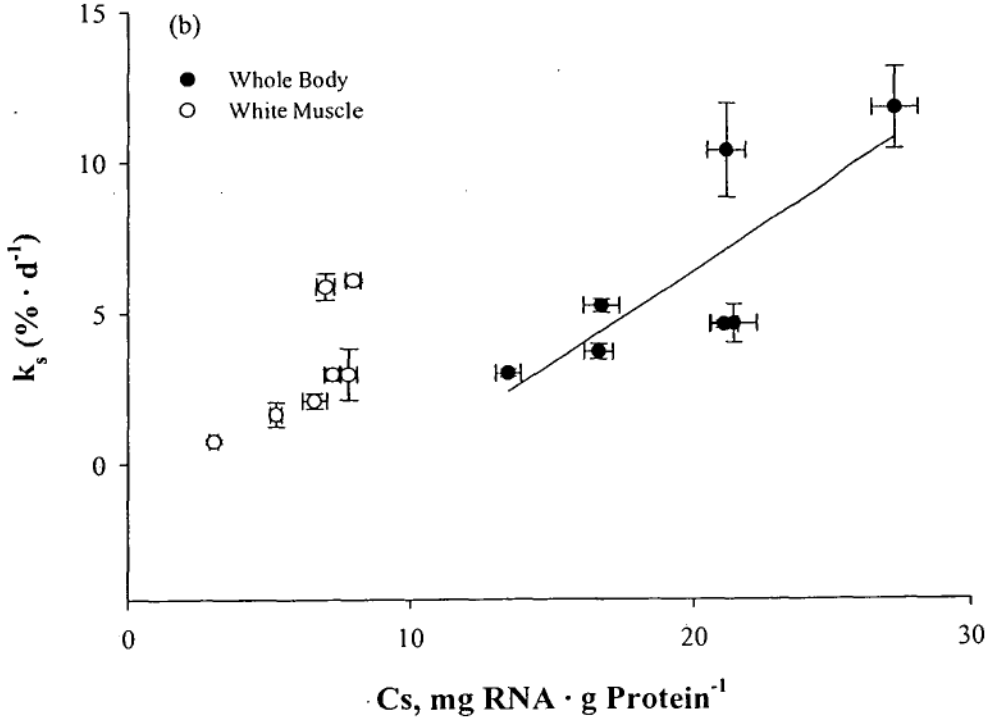
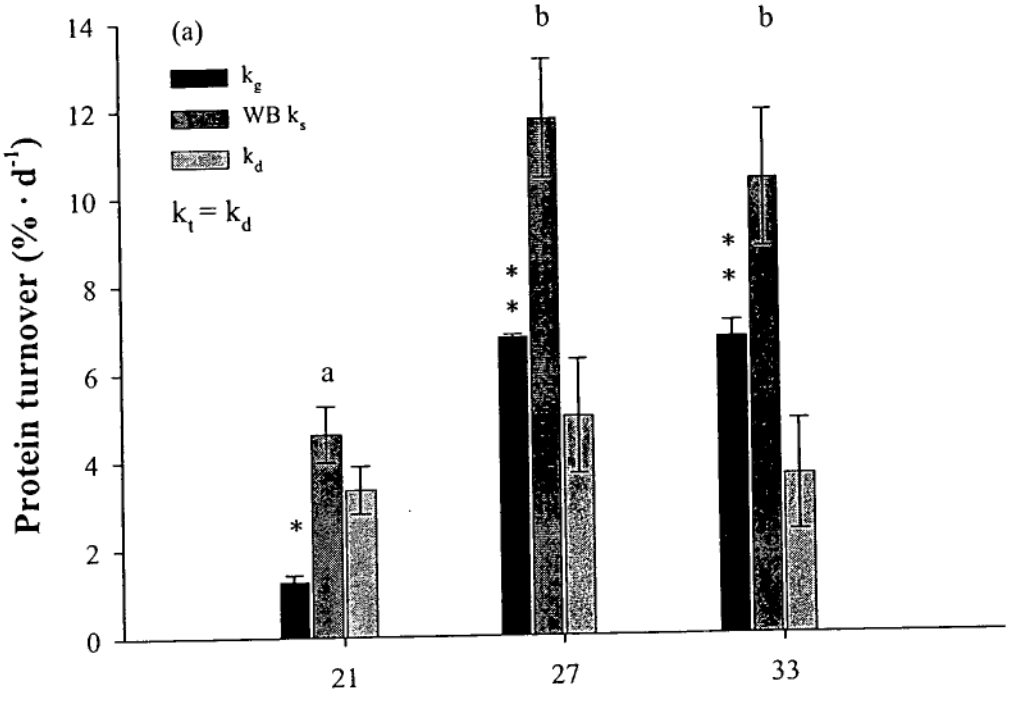
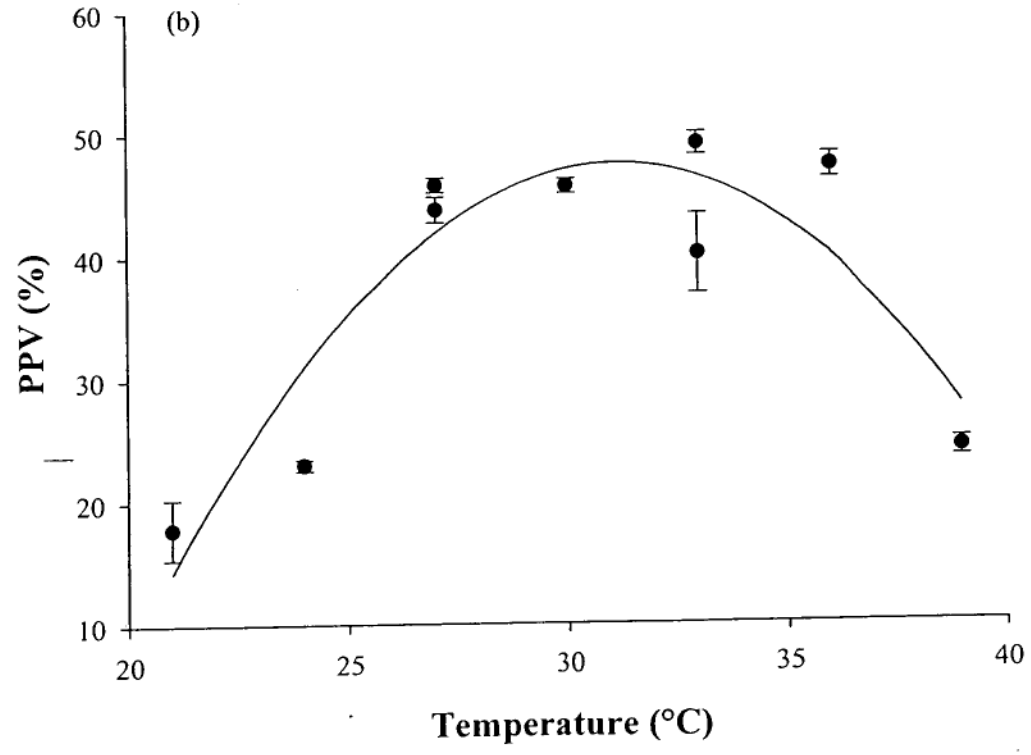

(b)
Whole Body
White Muscle
15
10
5
0
0
10
20
30
k_s (% · d^{-1})
Cs, mg RNA · g $Protein^{-1}$

Figure 7.5. (a) The whole body protein synthesis (WBk_s, $\%\cdot d^{-1}$), predicted protein growth (Pk_g, $\%\cdot d^{-1}$) and the predicted protein degradation (Pk_d, $\%\cdot d^{-1}$) to estimate protein turnover at temperatures of 21, 27 and 33°C. Significant differences between temperatures for WBk_s were indicated by different letters and significant differences between temperature for Pk_g were identified by asterisks (*, $p<0.05$). No differences occurred between Pk_d at any temperature tested. (b) The relationship temperature (°C) and the productive protein value (PPV, %) was best described by the equation, $PPV= -265.05+20.06T-0.3T^2$ ($r2=0.824$, $n= 9$, $p=0.005$).


(a)
k_g
WB k_s
k_d
$k_t = k_d$
Protein turnover (% · d^{-1})
14
12
10
8
6
4
2
0
a
b
b
*
**
**
21
27
33


(b)
PPV (%)
60
50
40
30
20
10
20
25
30
35
40
Temperature (°C)

7.3. REFERENCES

Carter, C.G., Houlihan, D.F., Brechin, J., McCarthy, I.D., 1993. The relationship between protein intake and protein accretion, synthesis and retention efficiency for individual grass carp, *Ctenopharyngodon idella* (Valenciennes). Canadian Journal of Zoology 71, 392-400.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod. Canadian Journal of Zoology 70, 2095-2102.

Houlihan, D.F., Mathers, E.M., Foster, A., 1993. Biochemical correlates of growth rate in fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 45-68.

Houlihan, D.F., Carter, C.G., McCarthy, I., 1995. Protein synthesis in fish. In: Hochachka, P., Mommsen, P. (Eds.), Biochemistry and Molecular Biology of Fishes, pp. 191-220.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences 45, 951-964.

Jobling, M., 1994. Fish Bioenergetics. Chapman and Hall, London, 309 pp.

Jobling, M., 1997. Temperature and growth: modulation of growth rate via temperature change. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp. 225-253.

Katersky, R.S., Carter, C.G., 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Lupatsch, I., Kissil, G.W. and Sklan, D., 2001. Optimization of feeding regimes for European sea bass *Dicentrarchus labrax*: a factorial approach. Aquaculture 202, 289-302.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., Burren, L.J., 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*: effects of age and temperature. Journal of Fish Biology 43, 245-263.

McCarthy, I.D., Moksness, E., Pavlov, D.A., Houlihan, D.F., 1999. Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Sciences 56, 231-241.

Meyer-Burgdorff, K.H., Rosenow, H., 1995. Protein turnover and energy metabolism in growing carp. 2. Influence of feeding level and protein:energy ratio. Journal of Animal Physiology and Animal Nutrition 73, 123-133.

Millward, D.J., 1989. The nutritional regulation of muscle growth and protein turnover. Aquaculture 79, 1-28.

Taylor, P.M., Brameld, J.M., 1999. Mechanisms and regulation of transcription and translation. In: Lobley, G.E., White, A., MacRae, J.C. (Eds.), Protein metabolism and nutrition. Wageningen Pers, Wageningen, pp. 25-50.

Williams, K., Barlow, C.G., Rodgers, L., Hockings, I., Agcorpra, C., Ruscoe, I., 2003. Asian seabass *Lates calcarifer* perform well when fed pelleted diets high in protein and lipid. Aquaculture 225, 191-206.

APPENDIX I

VALIDATION OF THE INCORPORATION OF ^{3}H-PHENYLALANINE

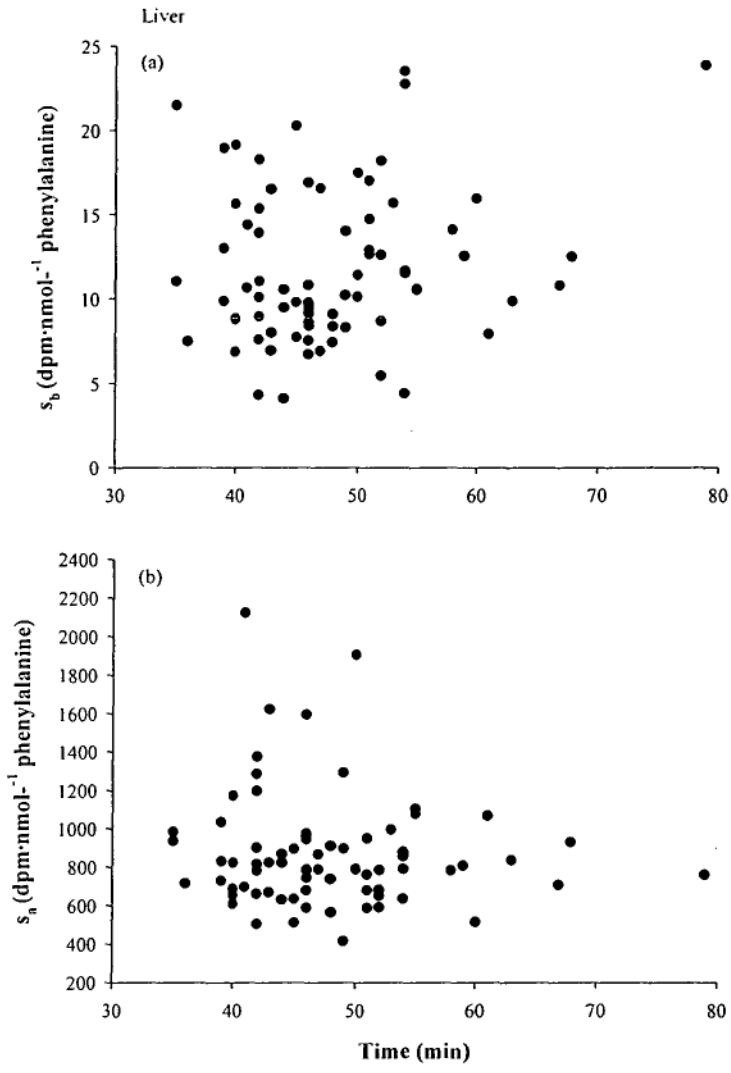


Figure AI.1. The incorporation of (a) the bound phenylalanine (S_b dpm·nmol^{-1} phenylalanine) and (b) the free pool (S_a dpm·nmol^{-1} phenylalanine) over the *in vivo* — incubation time in liver of juvenile barramundi across all temperatures tested (33-39°C). Neither the relationship between the bound (r^2=0.032; p=0.125; n=73) or free pool (r^2=0.011; p=0.370; n=73) phenylalanine and the incubation time were significant.

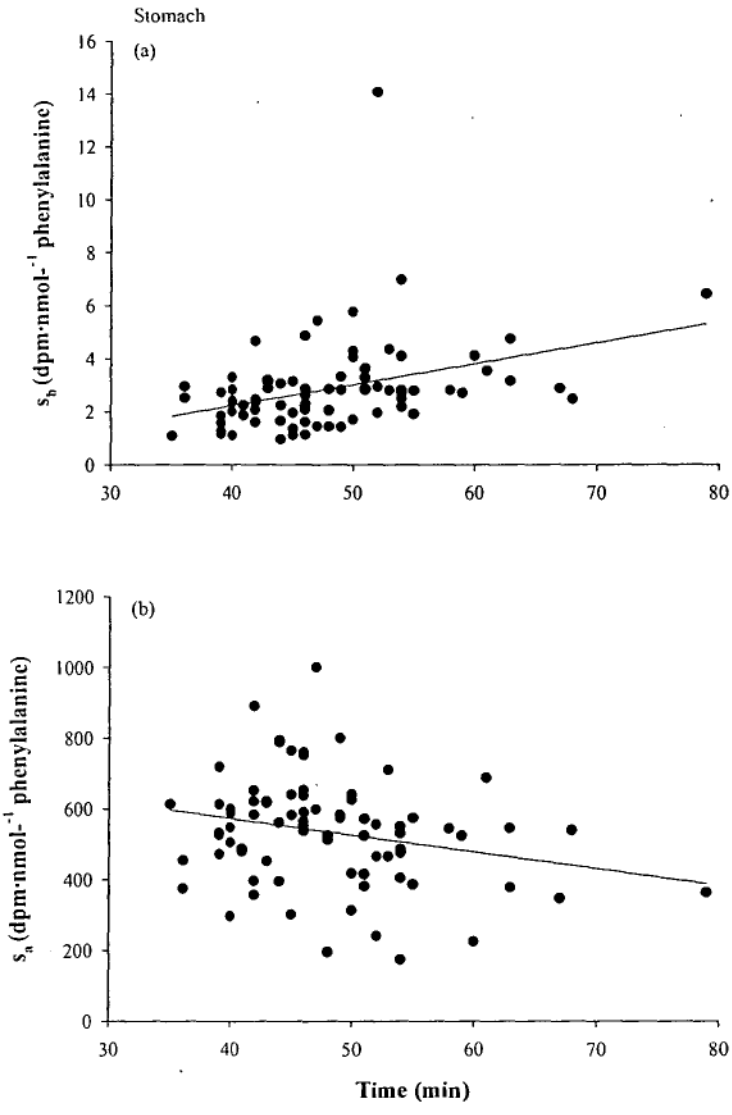


Figure AI.2. The incorporation of (a) the bound phenylalanine (S_b dpm·nmol^{-1} phenylalanine) and (b) the free pool (S_a dpm·nmol^{-1} phenylalanine) over the *in vivo* incubation time in stomach of juvenile barramundi across all temperatures tested (33-39°C). Both relationships between the bound and the free pool phenylalanine and the incubation time were significant. The relationship between the bound phenylalanine and the incubation time was described by the equation $S_b = 0.078T-0.905$ ($r^2=0.127$; $p=0.001$; $n=79$). The relationship between the free pool and the incubation time was expressed as, $S_a = -4.73T+762.16$ ($r^2=0.061$; $p=0.028$; $n=79$).

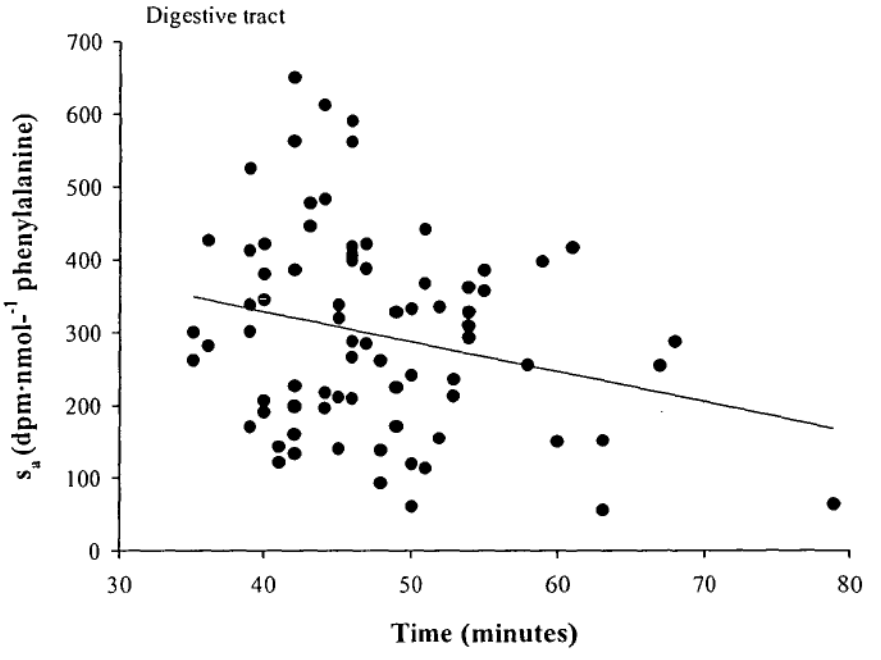


Figure AI.3. The relationship between the digestive tract free pool (S_a dpm·nmol^{-1} phenylalanine) over the *in vivo* incubation for juvenile barramundi across all temperatures tested (33-39°C). The relationship between the free pool and the incubation time was significant and negative and was best described by the equation $S_a = -4.11T+492.99$ ($r^2=0.060$; $p=0.032$; $n=76$).